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TECHNOLOGY UTILIZATION

ADVANCES IN STERILIZATION AND DECONTAMINATION

A SURVEY



NATIONAL AERONAUTICS AND SPACE ADMINISTRATION

200

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ADVANCES IN STERILIZATION AND DECONTAMINATION

A SURVEY

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Langley Research Center
Hampton, Virginia

Prepared under Contract NAS1-14209

by
The Bionetics Corporation ✓
Hampton, Virginia

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Foreword

The advent of space exploration has brought with it requirements for new and sometimes exotic ideas. The concept of "quarantining" a planet, for example, is at first a mind-boggling thought. However, considering that the evidence of a nonterrestrial life form could be irretrievably lost by inadvertent interaction with a terrestrial life form, protection against biological contamination is not such a strange idea. In fact, it becomes an objective of profound importance. The United States and other nations interested in space exploration agreed in the early 1960's that the search for extraterrestrial life forms was a primary objective and that special precautions had to be taken to prevent contamination of bodies in our solar system, which are considered possible habitats of life. A second area of concern was (and is) protection of the Earth from possible infection by extraterrestrial organisms.

Implementation of these concepts was made very difficult because of our gross ignorance about the nature of nonterrestrial habitats. We had no idea if nonterrestrial life existed, what it might be like, or how it might interact with terrestrial life forms either on its own ground or on Earth. In any case, a decision was made to protect the unknown planets, because of the enormous importance of the possible discovery of extraterrestrial life.

Thus, we were suddenly faced with such questions as:

1. How do you sterilize a spacecraft?
2. How do you keep it sterile?
3. How do you keep it at peak performance after sterilization?
4. How do you protect the Earth against an unknown organism?
5. How do you prove that an alien organism presents no hazard to a terrestrial organism or its environment?

These and a multitude of similar questions have occupied the time and attention of a number of scientists and engineers over the past 15 years and, in general, we now have answers to such questions. In the case of Viking, we actually designed, developed, and very successfully flew two sterilized landers to Mars.

We look ahead now to further exploration of the universe with confidence in our ability to protect the planetary environments involved, including our own. We also look forward to transferring the marvelous technology we discovered in our planetary quest thus far to Earth's industries for the benefit of mankind.

*Dr. Richard S. Young
Planetary Quarantine Officer, NASA*

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CHAPTER 1

Introduction

*The Antiseptic Baby and the Prophylactic Pup
Were playing in the garden when the Bunny gamboled
up;
They looked upon the Creature with a loathing
undisguised;—
It wasn't Disinfected and it wasn't Sterilized.*

Strictly Germ-Proof, Stanza I
Arthur Guiterman (1871-1943)

Sprung from Guiterman's fantasy garden, the loathsome Bunny visibly threatened the Baby and the Pup. Today, the creatures we see—and often don't see—in man's world are microbes that multiply more rapidly than rabbits and cause great concern to everyone, particularly to space explorers and to the food, medical, pharmaceutical, cosmetic, paint, and dry-cleaning industries. We are still babes and pups in an unclean world, unable to sterilize or disinfect it completely.

Many historical developments have contributed to the current knowledge of sterilization. The concept of decontamination started with the ancient Romans and Greeks, who used sulfur to fumigate and oils to embalm their dead. Aristotle advised Alexander the Great to have his armies boil their drinking water and bury their wastes. Even the Bible prescribes methods for handling and burning a diseased person's clothing. Louis Pasteur, Robert Koch, and other pioneers studied the relationships among microorganisms, food preservation, and disease; and Joseph Lister reduced the high death rates from surgery by using a germicidal chemical. The efforts of these scientists are recognized as the fundamental beginnings of sterilization and decontamination. Sterilization, simply stated, is the destruction of all forms of life (bacteria, yeast, fungi, and viruses); decontamination is the reduction in their number.

The purpose of this publication is to discuss the recent technical advances which have been made in

the field of sterilization and decontamination and their applicability to private and commercial interests. Government-sponsored programs by the National Aeronautics and Space Administration (NASA) produced the bulk of material presented in this survey. The summary of past and current research discussed is only detailed enough to enhance an effective transfer of technology from NASA to potential users.

NASA'S INTEREST IN STERILIZATION

Under funding by NASA contracts and grants during the past seventeen years, new technologies of sterilization and decontamination and the concomitant sterilizable space hardware have been developed, which also have wide application to many nonaerospace industries (figure 1). The investigations were stimulated by the search for extraterrestrial life, which has been one of the major scientific objectives of NASA's planetary exploration program. This objective, together with concern for the integrity of the planetary environments, resulted in the establishment of an international policy for quarantine of the extraterrestrial bodies against contamination of Earth origin. This policy necessitated the sterilization of certain spacecraft.

The spacecraft sterilization program dates back to 1960 with the effort to sterilize the second series of Ranger Lunar exploratory vehicles. At that time, space hardware was not designed to survive any

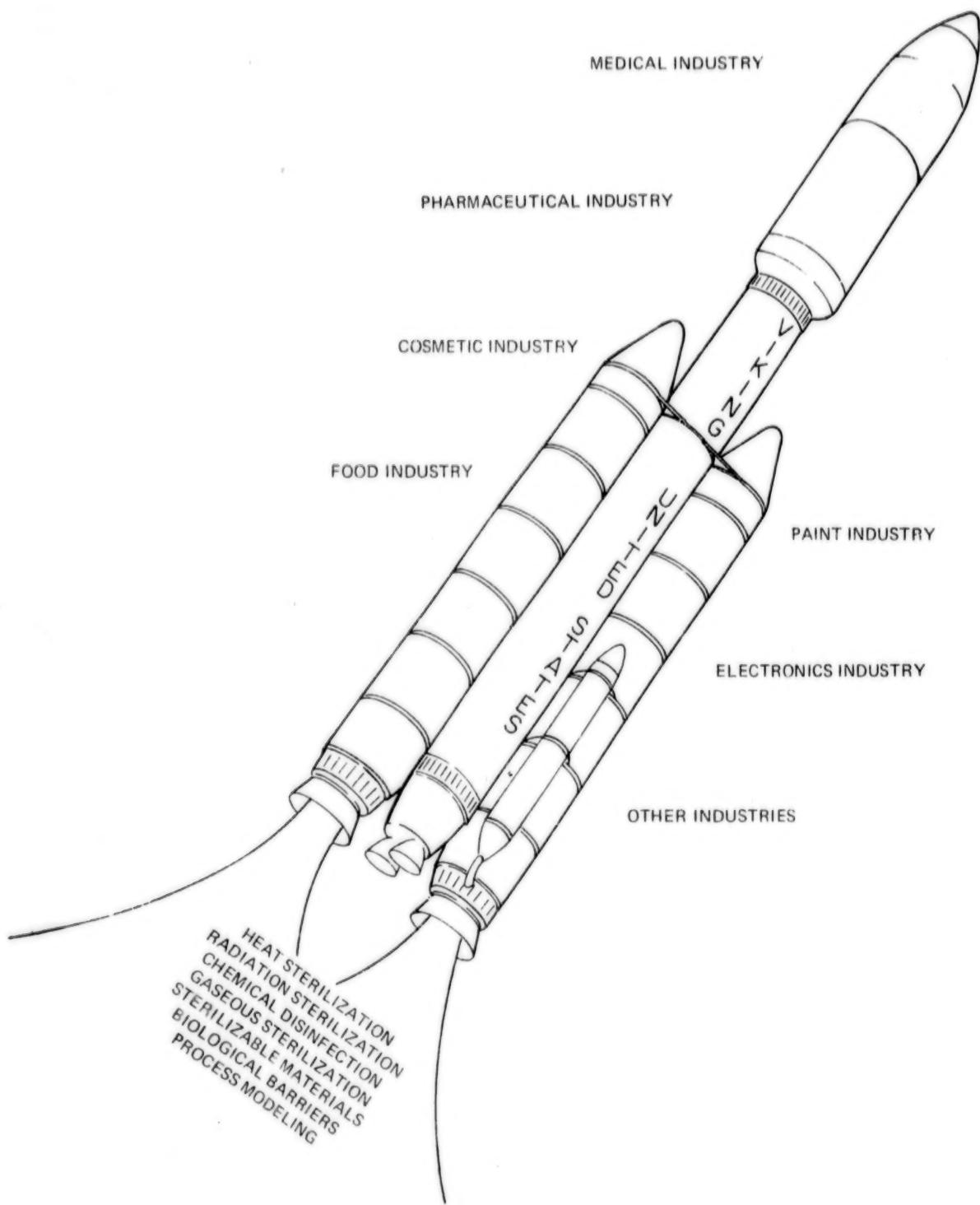


FIGURE 1. – Nonaerospace applications of NASA-sponsored research on sterilization and decontamination techniques.

sterilization techniques, particularly the destructive heat (24 hours at 125°C) imposed on some of the Ranger vehicles. Naturally, equipment failures resulted. Recognition that a problem existed led to the establishment by NASA of a major research program to determine possible sterilization and decontamination methods suitable for space hardware, and which would also protect Earth from "back" contamination from other planets.

The NASA research and development programs were undertaken primarily to advance the state-of-the-art sufficiently to permit sterilization of space vehicles without impairing their operational capacities. Particular success was achieved in advancing the technology of dry-heat sterilization, the method used to sterilize the two Viking Lander Capsules (VLC-1 and VLC-2) launched in 1975 that landed on the surface of Mars. Other advanced sterilization techniques such as thermoradiation and plasma cleaning were also developed; in addition to the development of new instrumentation, sterilizable parts, and clean room technology. These advancements have not only increased the pool of scientific knowledge and created new technologies, but in several cases have also opened up new pathways in the understanding of biological systems.

Specifically, the topics surveyed in this publication are:

- Current methods and concepts in sterilization and decontamination: heat sterilization, radiation sterilization, chemical disinfection, and gaseous sterilization
- Recent allied NASA technological developments: sterilizable materials, parts, and components, and biological barriers for aseptic assembly
- Thermal process calculations and modeling: analytical models.

CURRENT METHODS AND CONCEPTS IN STERILIZATION AND DECONTAMINATION

Heat Sterilization

Heat sterilization destroys the reproductive and metabolic activity of microorganisms. Two techniques of applying heat are discussed: (1) moist heat (steam under pressure); and (2) dry heat (a condition in which the air or inert gas contains little moisture—or less than 100 percent relative humidity—and the temperature

exceeds 100°C). Incineration will not be discussed, since the purpose of sterilization and decontamination is to destroy the contaminating organisms and not the infected article.

Moist- and dry-heat sterilization processes penetrate the surface of some solid items so that the interior and exterior can be rendered sterile. Moist heat is a reliable and efficient method of sterilization used in many industries. This method is used to sterilize items that are not altered to an unacceptable level in the process, or when deterioration due to moisture is not a problem. For example, hospitals use moist heat to sterilize surgical implements, linens, glassware, and similar articles. The food industry processes canned goods this way, and microbiological laboratories and the pharmaceutical industry also use the moist-heat sterilization technique.

Dry heat, in air or inert gas, is equally reliable though not as rapid as moist heat. It is used to sterilize items that are moisture sensitive and heat resistant.

Because some spacecraft parts are moisture sensitive, the NASA program ruled out the moist-heat process in its search for techniques to sterilize spacecraft. The choice quickly narrowed from alternative methods of heat, gas, and radiation to dry heat. The program that followed had two objectives: (1) to analyze materials, parts, and components to be sterilized; and (2) to develop an understanding of the biological and physical factors involved.

The increased understanding of the resistance of microorganisms to thermal destruction has led to the development of highly controllable sterilization processes. For example, processes have been developed to destroy even very resistant encapsulated microorganisms, caught in soil or plastic, or between mated surfaces. Through these NASA developments, non-aerospace industries can be assured that complex, moisture-sensitive devices such as electronic equipment can be dry-heat sterilized.

In addition, sterilization processes are being developed to kill so-called "hardy" psychrophilic microorganisms, which have recently been defined. This will be of great advantage to the food industry; because these microorganisms grow at low temperatures and over a broad temperature range (30°C to 55°C). Because of their extreme adaptability, they might thrive in such hostile environments as Mars, and will be significant in future flights to the colder planets of Jupiter and Saturn.

Radiation Sterilization

The early research efforts subjecting spacecraft hardware to dry heat identified many components, such as batteries and electronic parts, that were extremely susceptible to thermal deterioration. In an attempt to solve this problem, radiation and thermoradiation processes were investigated.

Radiation can destroy a microorganism's ability to reproduce or propagate. Beta rays, gamma rays, and X-rays have been used for this purpose because they can penetrate solid materials. Beta and gamma radiations are used to sterilize heat-sensitive plastics such as syringes, catheters, needles, catgut sutures, and artificial heart valves. They are also used to sterilize pharmaceuticals, blood products, and vaccines.

Thermoradiation sterilization is the simultaneous application of dry heat and ionizing radiation. This method reduces material or product deterioration, because the combined process of heat and radiation allows temperatures and dose rates to be lower than they would be for either process alone. For example, to accomplish sterilization by dry heat in a reasonable period of time requires a temperature in the range of 110°C to 125°C. Thermoradiation, however, can use temperatures between 60°C and 125°C with low-dose gamma rays to accomplish sterilization.

Thermoradiation is a recent development, and its potential applications are vast. Thermoradiation research includes: sewage sludge pathogen reduction; cancer therapy; chemical detoxification of liquid pollutants; and the sterilization of food, water, and pharmaceuticals. With thermoradiation, complex instruments such as cardiac-flow monitors, blood oxygenators, and kidney machines can be sterilized without operational performance losses.

Chemical Disinfection

The treatment of surfaces and solutions with chemical compounds to destroy microorganisms is known as chemical disinfection. Chemical disinfectants are widely used in hospitals, motels, hotels, and in the paint, dairy, food, and pharmaceutical industries. Current applications include use in skin antiseptics, body deodorants, swimming pools, sewage plants, and waste water effluents.

These disinfectants are normally used in solution, and are called bactericides because most spores and

certain other microorganisms are resistant to them. Bactericides that are currently in use include: alcohols, bisphenols, halogens, heavy metals, quarternary ammonium compounds, and formalin.

NASA originally became interested in bactericides (ethanol and isopropanol) to remove vegetative microorganisms from spacecraft surfaces. Research for more effective bactericides for use on spacecraft led to the discovery by Russian scientists of a hydrogen peroxide-surfactant solution. This bactericide was reported to leave no residues, to be nontoxic to humans when used at a recommended concentration, to not degrade most materials and, most importantly, to kill most spores.

Bactericides are used to keep water potable in spacecraft. For example, chlorine was used in the Apollo Command Module and iodine was used in the Lunar Lander. Present NASA research also includes the potential use of silver and other heavy metals to keep water potable during future extended space missions. These metals also are being explored for their use in skin ointments, antiseptic bandages, and antibacterial soaps.

One of the outstanding accomplishments of NASA-supported research was establishing the feasibility of self-sterilizing coatings. In this process, bactericides are incorporated within materials such as potting compounds to destroy the internal contaminants after curing. Tests using paraformaldehyde or a combination of ethylene oxide (ETO) and dimethyl sulfide in the polymer revealed that microorganisms can be destroyed. Applications are currently being investigated for prevention of microbial deterioration in the paint, plastic, and paper industries.

Gaseous Sterilization

Chemicals in a gaseous or vaporous state are used to destroy microorganisms on exposed surfaces, or in material that can be penetrated by the vapors.

The most common gas used for sterilization is ETO. NASA instituted the development of an ETO chamber which potentially can broaden the use of this method. Before the chamber was developed, metal foils of electronic parts were deteriorated by the sterilization process because of condensation of both ETO and water that caused corrosion. This is no longer a problem.

Other gases, such as beta-propiolactone (BPL) and formaldehyde, have been used as surface sterilants. In the past, formaldehyde was used to decontaminate rooms, but it left an irritating odor and residues that

were hard to remove. NASA-funded research has solved the problem by modifying an existing technique of neutralizing formaldehyde with ammonia. Under carefully controlled conditions and with adequate ventilation, the decontaminated room becomes odorless and usable shortly after neutralization.

RECENT ALLIED NASA TECHNOLOGICAL ADVANCEMENTS

Sterilizable Materials, Parts, and Components

With the development of new sterilization techniques developed during the Viking Program, NASA had to design compatible spacecraft equipment which could withstand heat sterilization and the space environment. As a result, materials, parts, and components created for specific needs and their reliability factors have been gleaned from the program.

For example, improved plastics and bonding agents were developed to withstand elevated temperatures. In developing the agents, it was found that they were strongly adhesive, flexible over wide temperature ranges, nonflammable, and exhibited good electrical properties. Although developed for another purpose, they have potential value for use in fire-retardant coatings on electronic devices.

An Electronic Parts Sterilization Program evaluated more than 72,000 separate parts. Over 655 million parts-test hours had been conducted when the program was completed, resulting in a list of parts that tolerate heat sterilization. For the Viking Mars Landers, components such as tape recorders, inertial instruments, batteries, and computer subsystems were developed to withstand the heat-sterilization cycle without significant loss in operational performance or reliability.

This information will enable future manufacturers, whose products require sterilization or decontamination, to select specific materials, parts, or components to fit their needs. Some of the items that can be sterilized today include implantable biotelemetry systems, heart pacemakers, and hospital operating room equipment.

Biological Barriers for Aseptic Assembly

Aseptic assembly programs range from the glove-box assembly of miniature electronic devices to a full-sized facility for the sterile assembly of spacecraft.

The technological advancements have been numerous and diverse. For example, a bioisolation suit completely enshrouds an individual like a "glove-suit." He is able to remain isolated, work in his specific area, yet still perform complex and delicate assembly operations under sterile conditions. The development of a special insertion technique also permits the individual to transfer sterile objects from area to area without contamination. Additional developments related to this field are clean room technology, and contamination control procedures.

The laminar flow clean room, unlike many other biological barriers, has self-cleaning capabilities. Airborne particles in the clean room are carried out of the room by a downward flow of air. The laminar downflow clean room provides the additional advantage of a dual barrier. It excludes external contamination from the room and internal room barriers prevent cross-contamination within the clean room. The principal function of a clean room is to control airborne particles by preventing them from entering an area or enclosure. This is accomplished by passing all the air through a highly efficient filter system. The air flows in one direction at a given speed to remove generated airborne particles and to prevent a buildup of contaminants in the clean room.

Clean room technology and the development of sterile insertion and assembly techniques presage a whole new vista for hospital operating rooms and patient care practice. They are currently used in the treatment of cancer patients, the protective isolation of burn patients, and the treatment of hypersensitivity. Sterile insertion and assembly techniques permit the safe production, packaging, and distribution of drugs, pharmaceuticals, and cosmetics. They also protect researchers from handling infectious disease agents, and from "back" planetary contamination.

THERMAL PROCESS CALCULATIONS AND MODELING

Analytical Models

As sterilization methods evolved, people in the space industry recognized that consideration of the basic thermal sterilization calculations used by the food industry might lead to further mathematical analyses and modeling of heat processes in general. Modeling, in simple terms, is the integration of several mathematical expressions that describe and predict physical, chemical, or biological processes.

Space researchers found that the food industry process (canning) retained food nutrients, while destroying microorganisms that caused food spoilage. After repeated successes—numbering in the millions—the desired food sterilization process had become predictable and consistent; however, the space industry was limited to few trials with a requirement for foolproof results. Errors had to be ruled out. The problem was to sterilize without losing hardware reliability due to deterioration from heat exposure. If the spacecraft were overtreated, reliability would be lost. If the spacecraft were undertreated, the search for extraterrestrial life would be jeopardized because the earthly contaminants would not have been destroyed. Facts were fed into computers, and programs were designed to calculate refinements in the sterilization and decontamination processes. However, national planetary quarantine policies evolved in the late 1960's making it unnecessary for spacecraft to be truly sterile and permitting certain low levels of bioload on programs such as Viking. By then the term "sterilization" was built into the working vocabulary and, hence, sterilization as used by NASA and throughout much of this book refers to a large reduction of bioload but not necessarily to true sterilization.

The sterilization process, as it applies to modeling for the space industry, involves two systems. The first determines, by limited sampling, the number of contaminating microorganisms on the total spacecraft's interiors and exteriors. The second system determines the time and temperature required to sterilize the microorganisms, based on thermodynamic characteristics of the space hardware. Deeper understanding of sterilization processes accompanied the development of reliable models and resulted in increased predictability.

The contribution of NASA in this field ranges from a data storage, retrieval, and analysis system that estimates microbial contamination on spacecraft to thermal process models that predict time cycles for sterilizing space hardware. The computerization of a program to identify microorganisms found on spacecraft has proven to be not only time- and cost-effective but also relatively free from data-reduction error. The use of such modified systems to identify pathogenic agents in automated clinical laboratories is readily foreseeable. Applications of biological computer programs for specimen analysis, patient diagnosis, and perhaps treatment are already being explored.

THE VIKING PROJECT

At 5:22 p.m. on the 20th of August 1975, a new phase began in America's planetary exploration, the launch of the first of two Vikings, the first United States spacecraft intended to land on the surface of another planet—Mars. Previous United States missions to Mars (Mariners 4, 6, and 7) flew by the planet. They were followed by the Mariner 9 spacecraft that went into orbit around Mars.

The Viking project was oriented toward advancing man's knowledge of the atmosphere and surface of Mars by direct measurements. The project consisted of two separate launches. Each launch contained a Viking Orbiter and a Viking Lander. Figure 2 shows a complete Viking space vehicle, with the Lander and Orbiter shown in the very top section.

Shortly after reaching Mars orbit, the Viking Lander separated from the Viking Orbiter and descended to the surface of Mars. The Orbiter performed remote scientific experiments and served as a radio relay link between the lander and Earth.

Figure 3 is an artist's drawing illustrating the lander being separated from the orbiter, the heat shield separating from the lander, the lander parachute and descent retrorockets gently lowering the lander and, finally, the lander in position on the surface of Mars. The landed Viking Lander, illustrated in figure 4, performed numerous experiments including scooping soil and depositing soil samples into the complex, automated Viking chemical and biological laboratories.

A large portion of the work described in this book was aimed at reducing the chances of carrying terrestrial organisms to Mars, where they would possibly mix into martian soil and confuse the data of the Viking biology experiments. NASA met this requirement by dry-heat sterilizing the Viking Landers and then encapsulating them in a sealed, pressurized cannister (bioshield) until they left the Earth's biosphere. Figure 5 shows an exploded view of a Viking Lander, its parachute and entry heat shield, and the encapsulating bioshields.

As the Viking Lander hardware was being assembled at NASA's Kennedy Space Center, more than 5000 biological samples were assayed by contractors and government microbiologists. These assays were used to determine the microbial burden on each lander and, hence, the time and temperature required for the sterilization cycle.

INTRODUCTION

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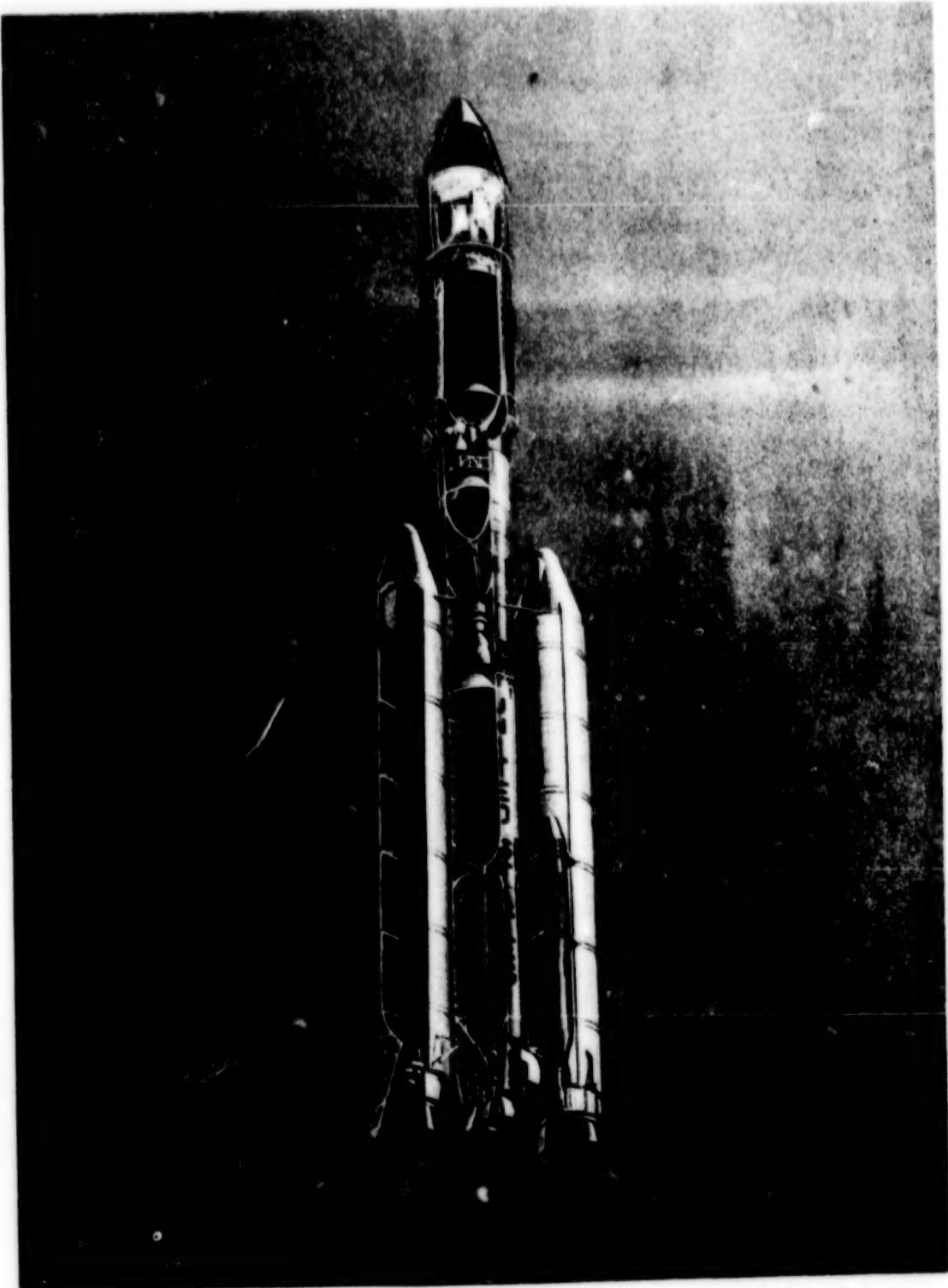


FIGURE 2. – Viking Space Vehicle.



FIGURE 3. – Viking Lander descent sequence.

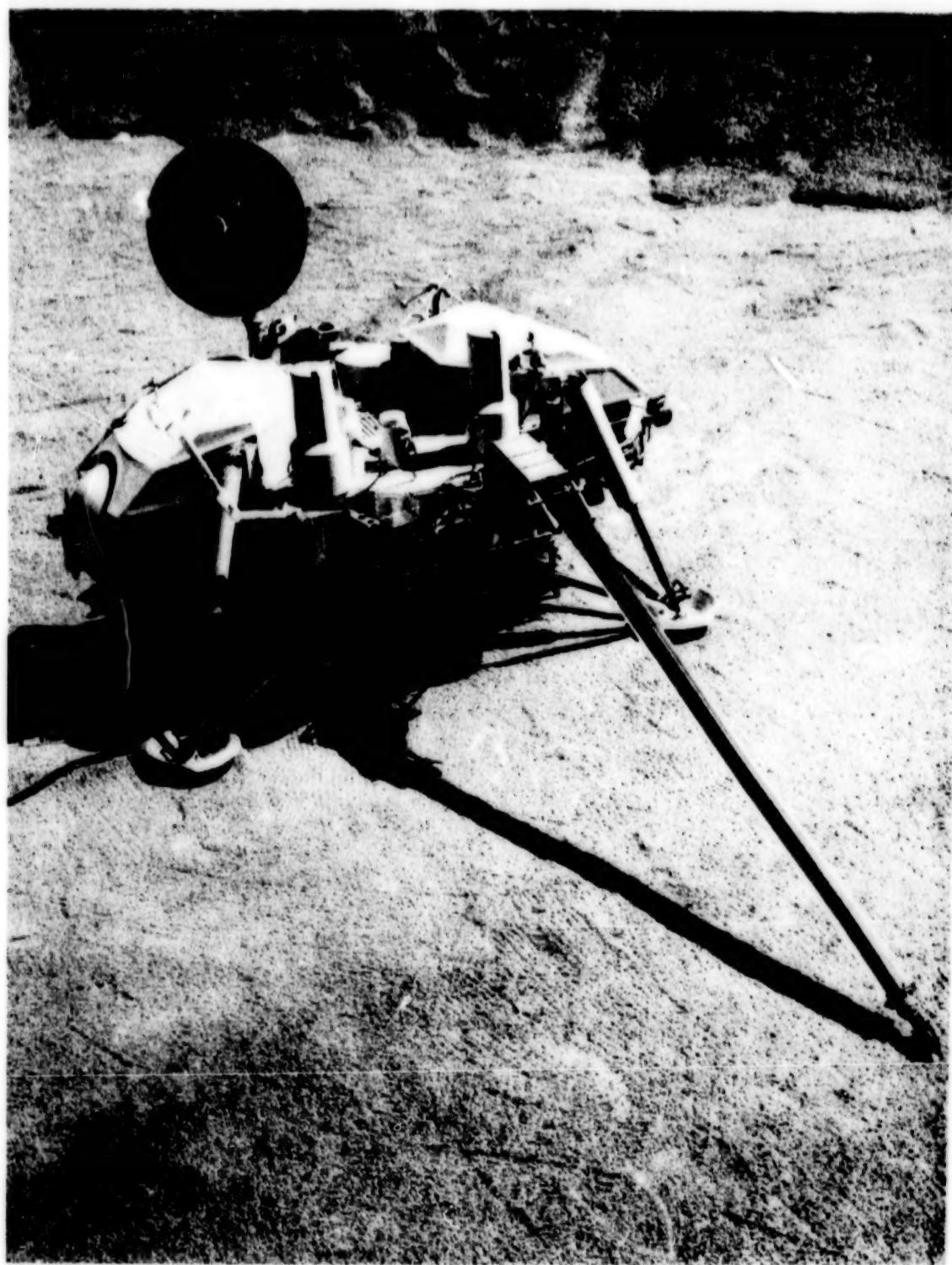


FIGURE 4. — Viking Lander.

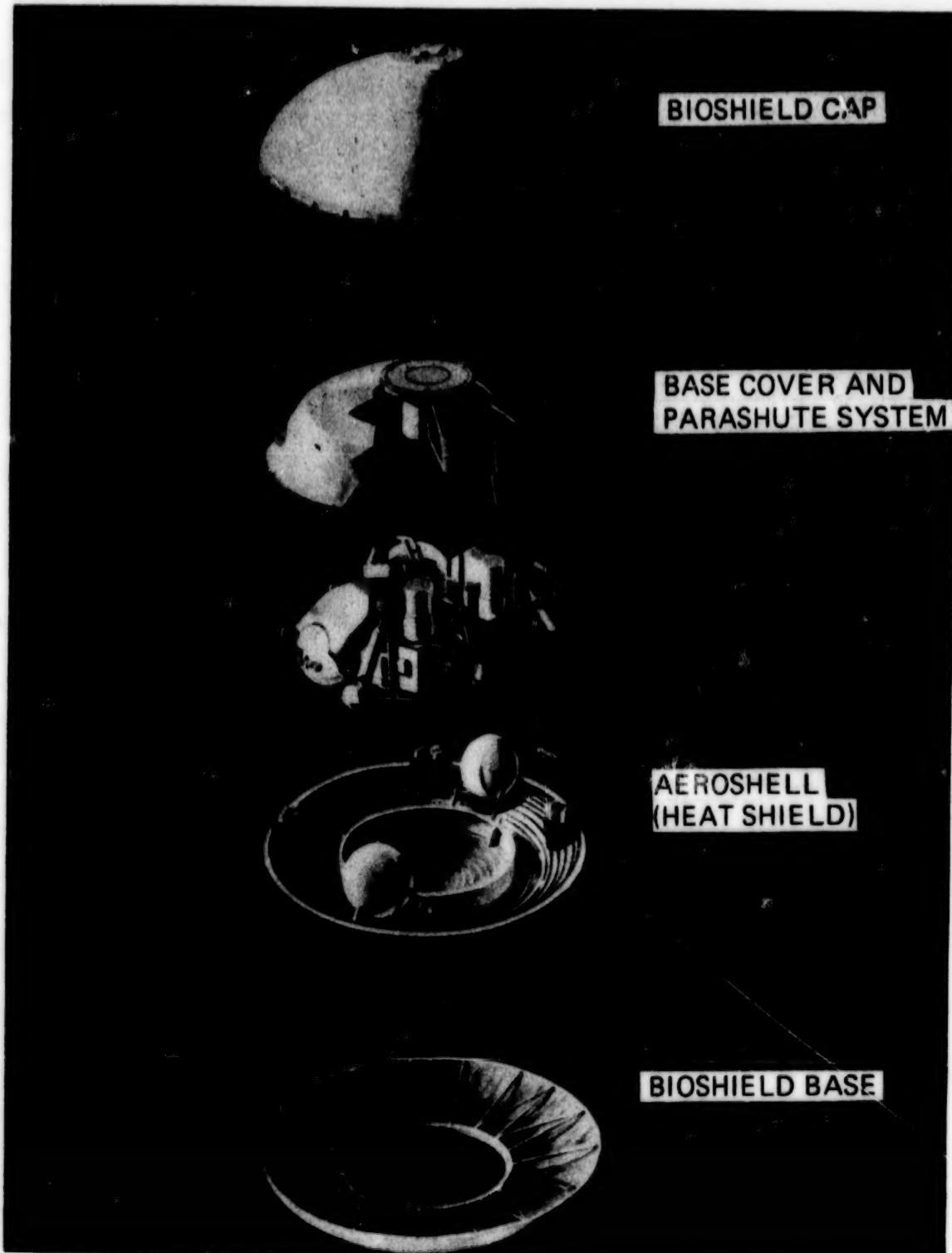


FIGURE 5. - Exploded view of encapsulated Lander capsule.

Figure 6 shows one of the encapsulated Viking Landers being placed in the sterilization oven at the beginning of the 40 to 50 hour sterilization cycle at a nominal temperature of 112°C. In figure 7 a sterilized Viking Lander is shown undergoing post-sterilization testing after removal from the oven. The flexible bioshield is fully pressurized. This positive pressure prevents recontamination of the lander; and it was continually maintained and monitored for the two months between sterilization and launch. In figure 8 the entire Viking spacecraft—enclosed in the Centaur Launch Vehicle Shroud—is being raised to the top of the Titan-Centaur Launch Vehicle. The long trailing hose includes the pressure makeup lines for the pressurized lander bioshield.

Figure 9 shows the launch of the second Viking space vehicle on September 9, 1975. Viking I was placed in orbit around Mars on June 20, 1976, and the Viking I Lander landed on the surface of Mars on July 20, 1976. Viking II was placed in orbit around Mars on August 7, 1976 and the Viking II Lander landed on the surface of Mars on September 3, 1976.

The success of the Viking Project represents an outstanding achievement in space exploration. Terrestrial microorganisms did not contaminate the Martian soil samples examined on Mars by Viking Landers I and II. The accomplishment of this planetary quarantine and the sterilization objectives, without

any damage to the Viking hardware, testifies to the success of the technologies discussed in the following chapters.

In summary, NASA has successfully developed the technologies needed to sterilize the most complex automated laboratory ever built by man. Hopefully, the descriptions of the NASA effort provided will prove useful in furthering commercial and private applications of sterilization and decontamination technology.

NASA'S CONTINUING ROLE

Although much work has been accomplished, much still needs to be done. NASA is continuing to sponsor projects that will further advance the field in line with its charter, which declares that its activities "should be devoted to peaceful purposes for the benefit of all mankind."

The Baby and the Pup recognized the Bunny and took actions to sterilize it to protect their garden. Protection of our earthly garden, and planetary gardens we have never seen, is the object of NASA's continuing research. The landing of the Vikings on Mars is the beginning of friendly visits to other planetary gardens, and signals improved methods of protection against microbial creatures in our own garden.

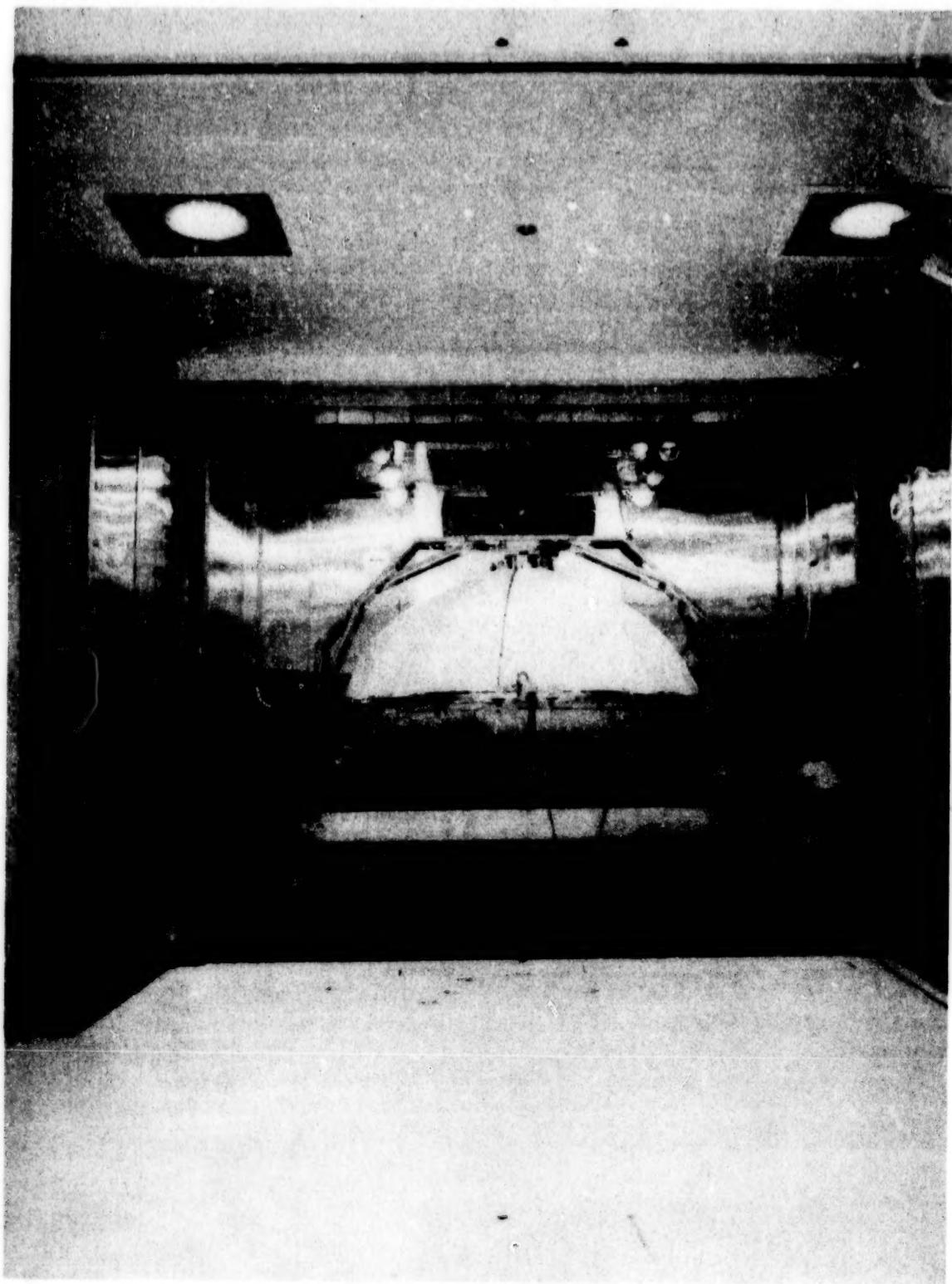


FIGURE 6. – A Viking Lander in the sterilization oven.

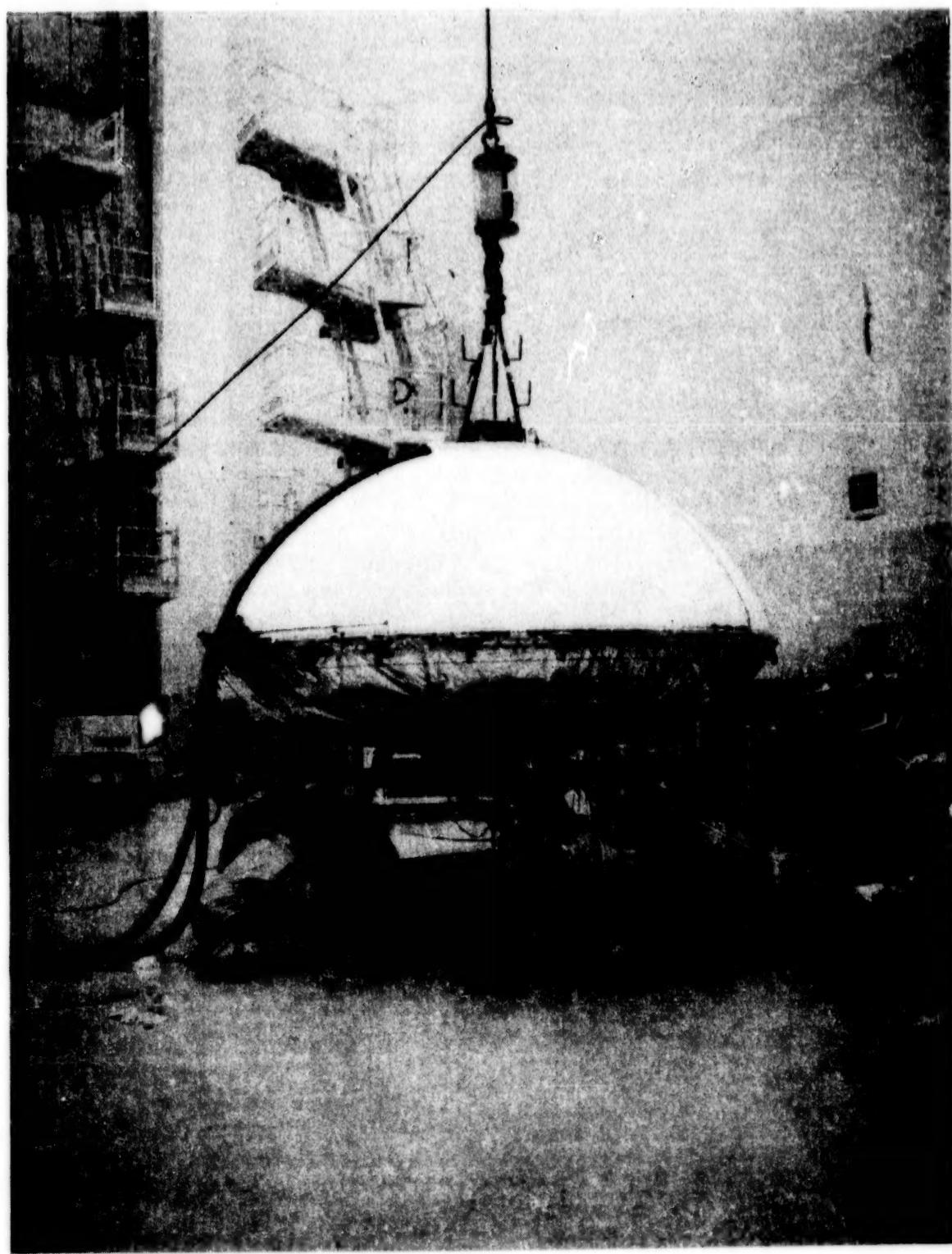


FIGURE 7. — Post-sterilization testing of one of the Viking Landers.

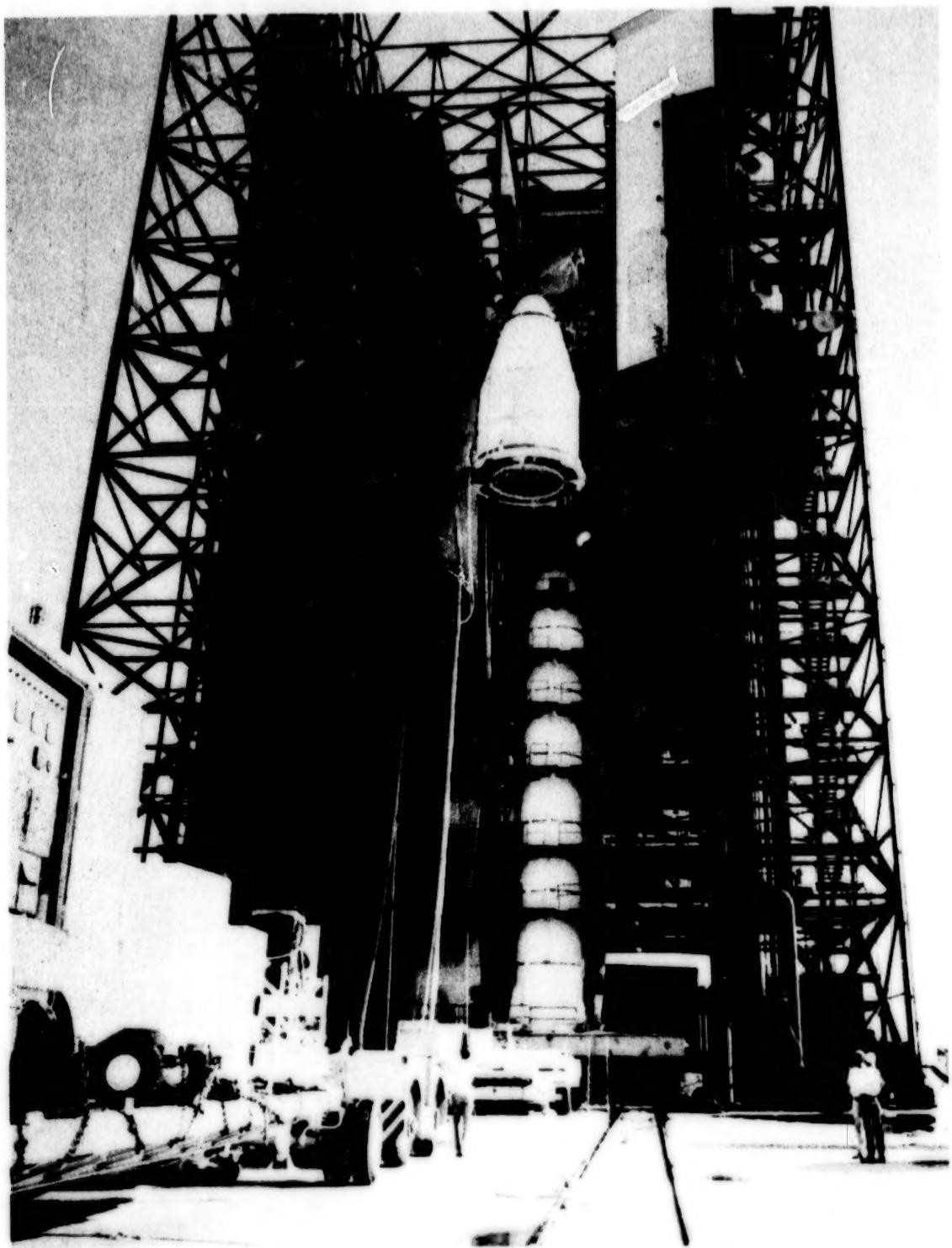


FIGURE 8. - Viking Spacecraft being lifted atop launch vehicle.



FIGURE 9.—Launch of the second Viking—September 9, 1975.

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PART 1

CURRENT METHODS AND CONCEPTS IN STERILIZATION AND DECONTAMINATION

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Heat Sterilization

*"They said it was a microbe and a Hot Bed of Disease
They steamed it in a vapor of a thousand-odd degrees"*

INTRODUCTION

The application of heat is one of the oldest techniques used for sterilization and decontamination. Boiling and burning have been used since Biblical times, and are still the most extensively used techniques today; however, our knowledge of the techniques and equipment used for sterilization is much more sophisticated. Today these techniques are called moist-heat sterilization and dry-heat sterilization.

Anyone who has sterilized a baby's bottle or done home canning is familiar with steam or moist-heat sterilization. Most people, without realizing it, are equally familiar with dry-heat sterilization. Anyone who has roasted a turkey, has dry-heat sterilized the bird's skin. When an uncovered turkey is put into the oven, it is exposed to a hot, dry environment—usually 350°F to 425°F (177°C to 218°C). As the bird cooks, the skin is first dehydrated and then begins to turn brown. As long as moisture remains in the skin, its temperature never gets above that of boiling water; because when the water evaporates, it draws heat energy from its surroundings that cools the surface of the skin. When the skin is totally dehydrated, it rapidly reaches the oven temperature and the hot oven air sterilizes the surface of the skin.

There are differences between dry-heat and moist-heat sterilization techniques. Sterilization by dry heat requires higher temperatures and longer exposure times than moist heat. These differences were quantified as early as 1880 by Koch, who called attention to the relative inefficiency of dry heat. In the ensuing years, only limited use was made of dry heat for sterilization purposes. Efforts were made primarily to understand, improve and apply the moist-heat process.

In 1960 the United States decided that the lunar and planetary environments must be protected from terrestrial contamination by its exploratory space vehicles (ref. 1). NASA recognized that for certain space vehicles to be sterilized, dry heat would have to be used. Moist heat could not be used because of the many problems (such as electrical shorts and corrosion) that moisture would cause in these complex spacecraft (ref. 2).

NASA attempted the dry-heat sterilization of Rangers 3, 4, and 5 (early lunar spacecraft) and uncovered many hardware problems that were the subject of investigations discussed in chapter 6 (ref. 3). (Later, in 1963, the sterilization requirement was removed from lunar spacecraft, but it has remained for planetary landing or impacting vehicles.)

In addition to uncovering hardware problems, the attempts to sterilize the Ranger vehicles by dry heat demonstrated that a large amount of microbiological research was needed to understand the dry-heat sterilization process. Little quantitative data existed at that time concerning the inactivation of microbial populations by dry heat. The data that did exist had been developed primarily for medical and laboratory applications. For example, dry heat had been used for sterilizing laboratory glassware, surgical instruments, catgut, petroleum jellies, and oils. However, the time-temperature cycles used for dry-heat sterilization varied widely. In 1964, a general review of dry-heat sterilization (ref. 4) cited the work of five different investigators, who reported greatly differing values for the resistance of *Bacillus anthracis* spores to dry heat.

With no data available on which to base the design of a dry-heat sterilization process, it became important for NASA to undertake a program to develop both

the information and technology necessary to ensure that a multimillion dollar spacecraft would be properly sterilized by dry heat and then perform its mission successfully. Vikings I and II, which were launched separately in 1975 and whose Landers I and II landed on Mars in 1976, were the first operational space vehicles to have been successfully sterilized by dry heat.

A PECULIAR PROBLEM—VERIFICATION OF STERILIZATION

In developing sterilization programs for the Viking Lander, NASA had to face up to the verification problem peculiar to spacecraft sterilization, and not generally encountered in commercial operations.

In commercial sterilization processes, sterility is verified by a combination of methods (Pflug, 1973): (1) the use of quality control checks and process records to verify that design conditions of microbial load are met and that the specified sterilization process is delivered; (2) use of chemical or biological monitors to verify the delivery of the sterilization process; and (3) testing of a representative number of the sterilized product, usually 20 or more samples from each lot.

Verification of sterilization by direct sample testing was neither practical nor economically feasible for the NASA Viking space vehicle, for only three or four vehicles were provided for each mission.

The use of biological or chemical indicators was also ruled out. Commercially used chemical indicators include a variety of products ranging from heat-sensitive tapes to small tubes of chemicals. Sterility is usually indicated by a change of color or a change of material from a solid to a liquid. Biological indicators take the form of bacterial cultures within sealed glass tubes or on paper strips in envelopes. The spore strips can be placed at any location, exposed, then aseptically transferred to a biological-growth medium to test the sterility of the spore strip. Since the spore strip contains a very high concentration of heat-resistant spores, if the strips are sterile, then by inference the unit being sterilized should also be sterile. A strain of *Bacillus stearothermophilus* spores is generally used as the biological indicator for moist-heat sterilization processes. *Bacillus subtilis* var. *niger* spores are generally used as the dry-heat biological sterility indicator.

Indicators could not be used to verify sterilization of the NASA Viking Landers; because each Viking Lander was encapsulated before sterilization in a bioshield that acted as biological barrier until it was separated after launch (fig. 3). This barrier protected the Lander from potential recontamination during post-sterilization and prelaunch activities; but also prevented the removal of potential biological or chemical indicators that might be used to verify sterility.

Therefore, the only approach left to NASA was to precisely design the sterilization cycle and then physically verify that the cycle had been delivered. This meant assuring that the physical and measurable parameters of time, temperature, and humidity obtained during the sterilization process resulted in the necessary reduction in bacterial population (ref. 5). This required experimentally determined data for the dry-heat resistance of organisms, an understanding of the factors of thermal resistance of microorganisms and the pertinent thermodynamic factors of space hardware, and the development of predictive models for microbial contamination. These models are discussed in chapter 8.

COMPARISON OF MOIST- AND DRY-HEAT STERILIZATION PROCESSES

Before discussing the factors affecting the dry-heat resistance of microorganisms, it is important to understand the basic characteristics of dry- and moist-heat sterilization.

It is common practice to divide heat sterilization into "wet-heat" and "dry-heat" sterilization processes. In wet-heat sterilization, water in the liquid state is present in the system. In dry heat sterilization, the quantity of water is not zero, but the substrate is not wet. Unless heroic measures are employed, there will always be some moisture present regardless of the system or the item to be sterilized. Therefore, dry-heat sterilization is a heat process in which the quantity of water in the system ranges from a relative humidity (RH) value near 0 percent to near 100 percent saturation. (Saturation, or 100 percent RH, is the condition necessary for wet-heat sterilization.)

One of the most significant discoveries affecting the industrial revolution and population dynamics of the 19th and 20th centuries was the first practical application of moist-heat sterilization. In France in 1809, Nicholas Appert developed the technique of preserving foods that today we call "canning." This

method of preserving food freed armies from their dependency upon fresh food supplies and negated the effect of the "scorched earth" tactic. It permitted preservation of harvest and assuaged the effects of "years of feast and years of famine." It enabled cities to grow because millions could be fed at great distances from the food production areas. It also opened the doors of international trade since foods grown in one nation could now be consumed and enjoyed in other nations.

Appert's original technique was to place food in glass jars that were in turn placed in boiling water for prolonged periods. Immediately after boiling, tight fitting cords were inserted into the opening in the jars. Only then was cooling permitted (ref. 6). It was not until the 1850's that the steam-pressure vessel was invented, and not until 1874 that the closed steam-pressure retort or autoclave was patented and came into use. Until then boiling was the only means of achieving moist-heat sterilization. Steam is a far more efficient heating agent than boiling water (ref. 7); and today the use of saturated steam under pressure for sterilization is almost universal. Autoclaves or retorts are commercially available in many sizes and degrees of sophistication. The smallest may be no more than an adaptation of a domestic pressure cooker. The sophisticated high-vacuum steam sterilizers, of the type shown in figure 10, contain automatic controls, steam pulsing systems, and precision monitoring instruments.

When steam at 121°C is brought into contact with a cooler material, for example at 80°C, heat is transferred to the colder material and raises its temperature. The steam condenses to water as heat is given up. This action continues until a temperature equilibrium has been reached, after which there is no more heat exchange or further condensation.

A range of standard cycles recommended for sterilization with steam under pressure are: 15 minutes at 121°C [15 pounds per sq. in. gage (psig)]; 10 minutes at 126°C (20 psig); and 3 minutes at 134°C (29.4 psig) (ref. 7). These sterilization cycles will result in the destruction of a million resistant *Bacillus stearothermophilus* spores. In dry air the process cycles necessary to obtain an equivalent destruction of these spores would be approximately 135 minutes at 121°C; 90 minutes at 125°C; and 35 minutes at 134°C.

Thus the steam autoclave is a far more efficient sterilizing machine than a dry-heat oven. At 121°C,

it takes nearly 2000 times as long to sterilize *B. subtilis* var. *niger* spores in hot air as it does in steam (ref. 7). The heat sterilization process, in which the gaseous heating medium is at relative humidities below 100 percent, is called "dry-heat sterilization." The gaseous heating medium is usually either air or nitrogen, but other gases such as helium and argon could be used. Even in this so-called dry-heat sterilization the gas and product contains a quantity of water. The amount of water present in the spores' environment prior to sterilization and the sterilizing gas itself may affect the ability of the organisms to survive dry-heat sterilization. (The influence of water on microbial death will be discussed extensively in the next section of this chapter.)

Equipment used for dry-heat sterilization includes ovens varying in size from a standard household range up to the sophisticated Jet Propulsion Laboratory oven shown in figure 11, which can handle a spacecraft 12 feet (3.6 meters) in diameter.

DRY-HEAT RESISTANCE OF MICROORGANISMS

Death of Microorganisms

Before determining those microorganisms that resist heat destruction, it is important to be able to distinguish microorganisms that die from those that survive.

In this era of external life support systems, determining when death occurs is a difficult problem. For the human organism it is a medical-legal problem and requires a medical-legal definition. It is even more difficult with microorganisms, since many of the obvious life signs of higher animals are not present in bacteria or their spores. Living or dead microorganisms may have the same physical appearance when directly observed under a microscope, and also may have similar reactions to chemicals such as stains.

A widely used practical criterion for the death of microorganisms (ref. 8) is the failure of the organism to reproduce when suitable conditions for reproduction are provided. Thus any organism that fails to show evidence of growth—usually the formation of a colony—when placed under adequate growth conditions is considered dead. When a viable microorganism is placed on or in a medium suitable for growth, it multiplies. When it has multiplied so that an entity is visible to the naked eye, it is referred to as a colony

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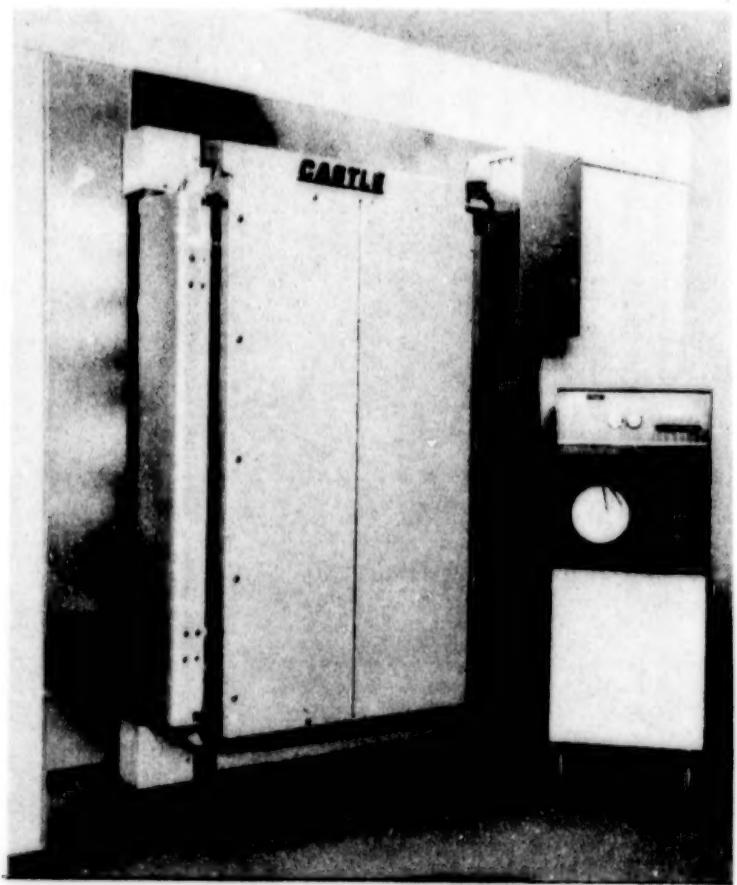
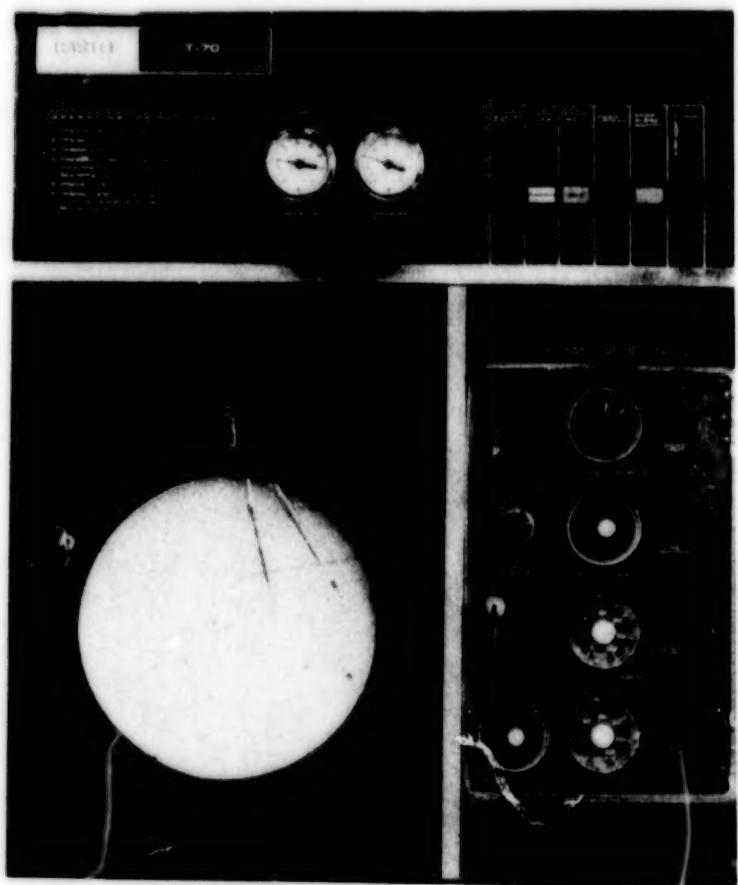


FIGURE 10. — High vacuum steam sterilizer.

HEAT STERILIZATION

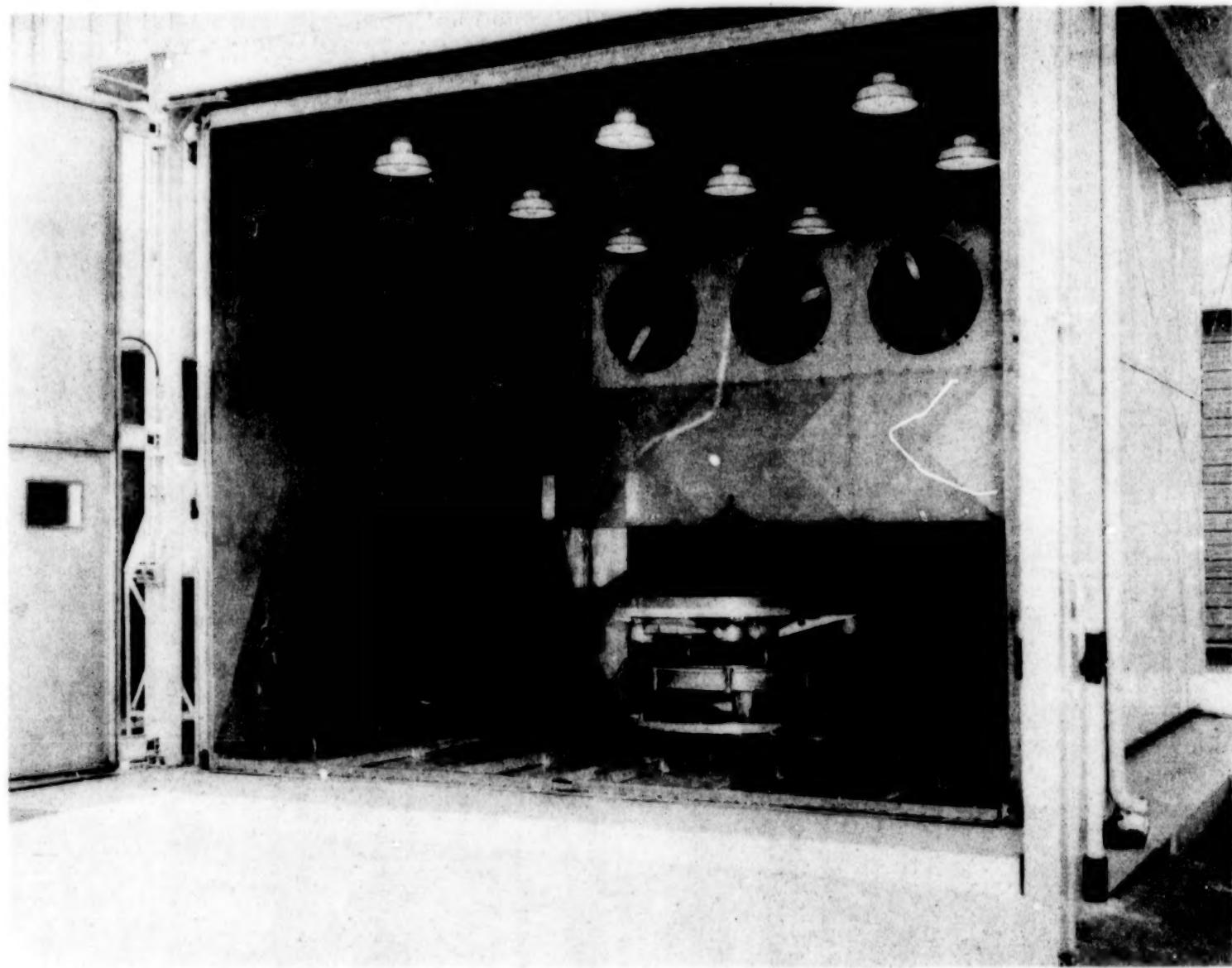


FIGURE 11. - JPL sterilization oven.

if on solid media, or by turbidity if in a liquid growth media. By this means the viable microorganism can be distinguished from the dead cell, which cannot multiply.

The lethal effect of heat is determined by exposing a microbial population to heat under known conditions and noting the number of organisms that survive the stress condition. This method is simple, but it is not without drawbacks. For example, those microorganisms that are unable to form colonies are assumed dead, which is not always true. Death is only one result of a complex series of molecular interactions; injury or sublethal damage is another result. In many cases, the nature of the growth medium and the incubation conditions will affect the ability of microorganisms to reproduce and therefore their measured response to a lethal agent.

Fortunately, despite these variances, the death of a population of microorganisms at elevated temperatures, in general, follows an orderly progression and can be shown to follow certain mathematical forms (refs. 9 and 10). In most cases, the relationship between the time of thermal exposure and the number of surviving microorganisms follows an exponential curve, indicating a constant death rate. In other words, the same percentage of viable cells will be inactivated in any unit of exposure time. If the logarithm of the number of survivors is plotted against time of exposure, as in figure 12, the relationship that results is a straight line. This is called a semilogarithmic survivor curve.

Not all reported experimental data form straight-line logarithmic survivor curves. Semilogarithmic survivors that are concave-upward, concave-downward, and sigmoid have been reported in the literature (ref. 10). These differences are to be expected since it is difficult to control the precise criteria necessary to experimentally demonstrate that microorganisms die exponentially. For example, all cells must be genetically, chemically, and physically homogenous, and all cells must be exposed to identical thermal environments and recovery methods. Otherwise, nonlinear survivor curves can result. For instance, a concave-upward survivor curve could be obtained from a microbial population that contains bacterial spores of varying thermal resistance, whereas a concave-downward curve should result from a severe clumping of the exposed cells (ref. 10).

Measurement of Heat Resistance

The D-value and z-value are two specific parameters that are used to describe and compare the thermal resistance of microorganisms. They are also used in designing sterilization processes. The D-value is a measure of the resistance of a suspension of microorganisms in a specific test system at a specific temperature. It is the "decimal reduction time," or the time at temperature T required to destroy 90 percent of the microbial population. It is the negative reciprocal of the slope of the straight-line, semi-logarithmic survivor curve. Numerically, the D-value is equal to the time it takes for the survivor curve to traverse one logarithmic cycle.

The z-value, in degrees of temperature, represents the rate of change of resistance (D-value) with temperature. It is the negative reciprocal of the slope of a thermal destruction curve (as shown in figure 13), which is a plot of the logarithm of the D-value vs. temperature (in degrees Celsius or Fahrenheit). Numerically, z is equal to the number of degrees, either Celsius or Fahrenheit, required for a thermal destruction curve to traverse one logarithmic cycle of D-values (ref. 11). The D- and z-values are affected by species and strain, the culture conditions under which the microorganisms are grown and recovered (such as age, growth, temperature, and nature of the medium), and the specific experimental conditions under which the data are obtained.

Design Basis for Dry-Heat Sterilization

In studies involving dry heat, the spores of *Bacillus subtilis* var. *niger* are usually used as the test or reference organism since they are relatively resistant when exposed to dry heat. Considering the thermal resistance of microorganisms normally found on spacecraft and realizing that it would be practically impossible to deal with all variations in thermal resistances, NASA accepted *Bacillus subtilis* var. *niger* as the representative organism for tests (ref. 12). This choice was not a "worst case" selection but a moderately conservative one. During the past decade, NASA sponsored a series of investigations using this representative organism.

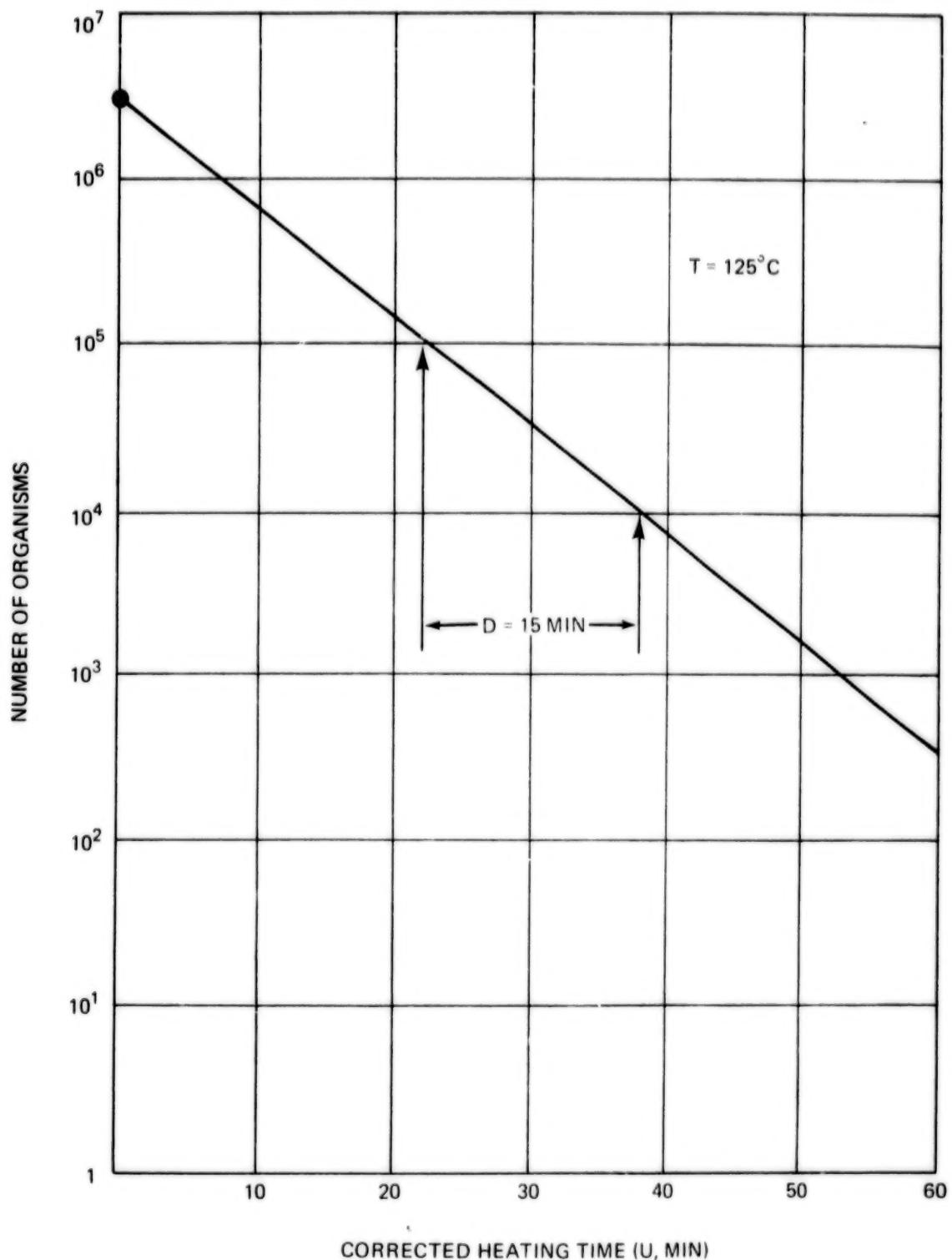


FIGURE 12. – Typical straight line semilogarithmic survivor curve for *Bacillus subtilis* var. *niger* spores on a stainless steel surface.

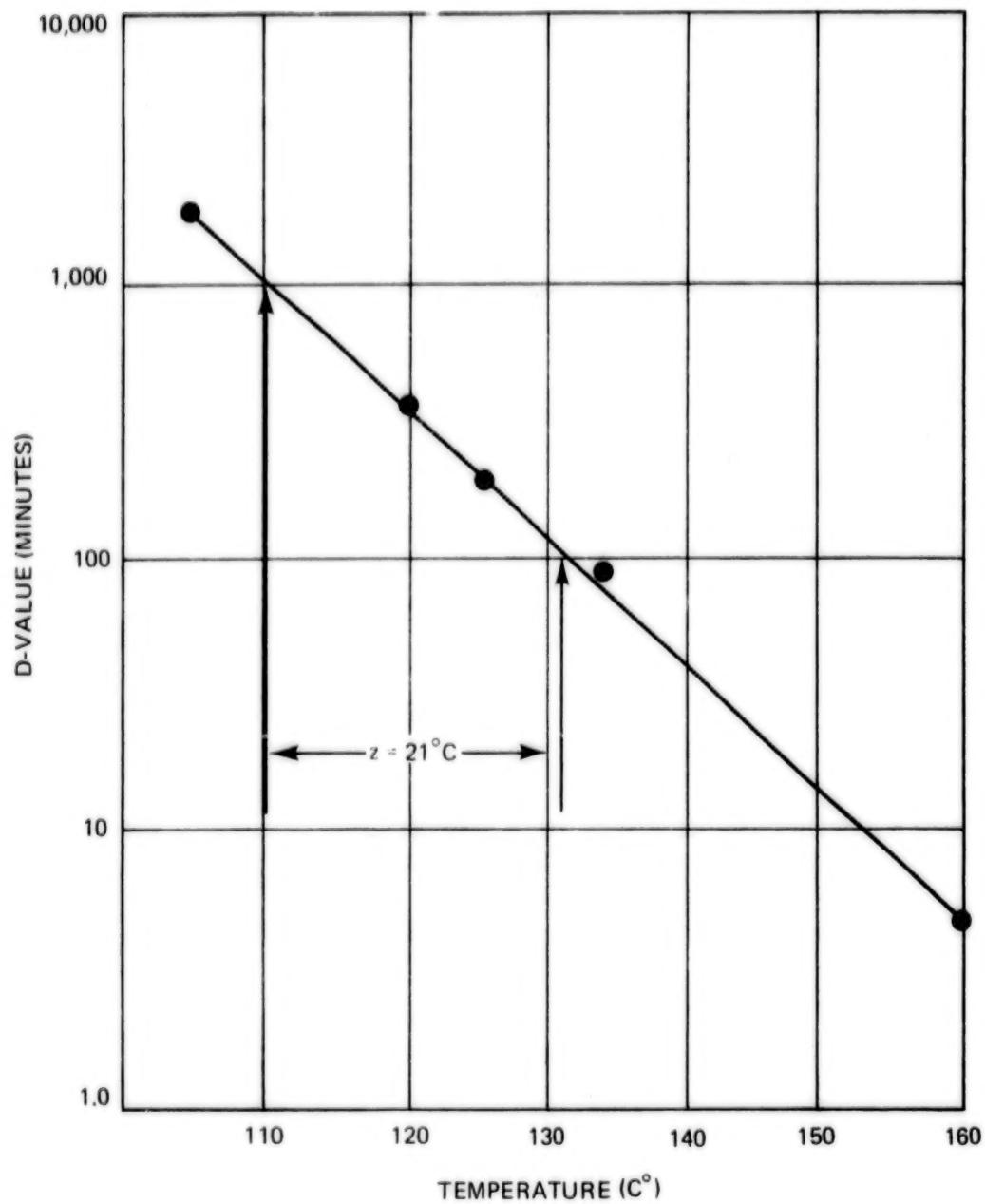


FIGURE 13. – Thermal destruction curve for *Bacillus subtilis* var. *niger* spores encapsulated in Lucite.

The NASA investigations were broad in scope. They covered the effects of culture conditions, including the investigation of the composition of sporulation and recovery media, temperature and times of incubation, and methods of storage of spore suspensions (refs. 13, 14, and 15). Numerous techniques were investigated and used to determine the best way of obtaining thermal resistance data (refs. 13, 16, 17, and 18). Complete spacecraft were assayed (refs. 19 and 20). Hundreds of cultures were identified and many D-values were determined to provide the necessary assurance that the selection of *Bacillus subtilis* var. *niger* as an organism was correct. Computer programs were developed for handling and analyzing the data (refs. 19 and 20).

The results of these studies verified earlier reports by showing that the D-values for bacterial spores subjected to dry heat varied greatly, depending on the experimental techniques used and the characteristics of materials on which or in which the spores were placed.

Since a space vehicle contains a multitude of different materials and configurations, it was an impossible task to determine the thermal resistance of microorganisms within each possible material and for each hardware configuration. The results of the NASA research program eased the problem. It was found that the differences in D-values were not solely due to the physical characteristics of the material, but rather to a combination of the spore condition and the characteristics of its microenvironment, particularly those that affect the rate of water transfer to and from the spores (refs. 16, 21, 22, 23, and 24).

FACTORS AFFECTING DRY-HEAT RESISTANCE

In discussing factors affecting the dry-heat resistance of spores, it is important to separate primary factors from secondary factors. Temperature, time, and water content are the three primary variables affecting the dry-heat resistance of microorganisms. All effects are a direct function of time; therefore, the effect of time will not be discussed further.

A discussion of the important secondary variables—open and closed systems, physical and chemical properties of the microorganisms and adjacent support; e.g., encapsulation, and the gas atmosphere—which play an important role in determining microbial water content during heating, will follow discussion of temperature and water content.

Temperature

Temperature, which is the measure of the heat energy level, is the most important variable in the dry-heat destruction of microorganisms. Its action is a function of time. Thermal destruction curves for *Bacillus subtilis* var. *niger* spores in five different systems (Data of Angelotti et al., 1968a) are shown in figure 14.

Dry-heat resistance values for several species of microorganisms are shown in table 1.

The z-value of the Bigelow (1921) model is one measure of change in the destruction rate with temperature. Reported z-values for dry-heat destruction of microbial spores range from about 15°C to 30°C (27°F to 54°F) with Q_{10} values of 4.6 to 2.2. In the temperature range of 105°C to 135°C, the average z-value is about 21°C, and Q_{10} is 3.0. A z-value of 21°C was adopted by NASA (1969). The z-value for microbial spores subjected to wet heat is in the range of 8°C to 10°C with Q_{10} values of 17.8 to 10. (It is this major difference in z or Q_{10} value that is the basis for the assumption that the mechanism of dry-heat destruction is different from the mechanism of wet-heat destruction.)

Water Content

Recently, evidence has shown that water has a direct influence on microbial resistance to destruction by dry heat. The effect of water on the dry-heat destruction rate of spores was first reported by Murrell and Scott (1957). This initial report has been supported by additional studies by Murrell and Scott (1966) and Angelotti et al. (1968a). In addition to these studies dealing directly with water and dry-heat destruction, there have been a large number of studies in recent years reporting dry-heat destruction rates for specific conditions: Pflug (1960); Jacobs et al. (1965); Pheil et al. (1967); Paik et al. (1967); Green et al. (1967); Fox and Pflug (1968); Silverman (1968); Hoffman et al. (1968); Bruch and Smith (1968); and Bond et al. (1970).

It appears that the dry-heat destruction rate of microbial spores is a function of the quantity of water in the cell at the time of heating, which is determined by the relative humidity of the atmosphere surrounding the cell. Therefore, the destruction rate will vary with the RH of the system; and the RH conditions must be specified, in addition to the

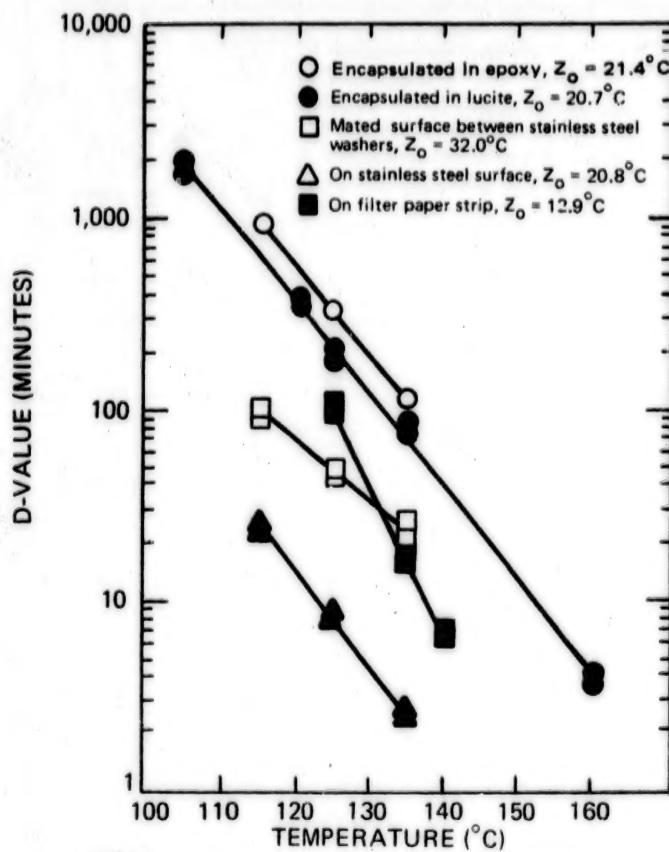


FIGURE 14. – Thermal destruction curves for *Bacillus subtilis* var. *niger* spores (Angelotti et al., 1968a).

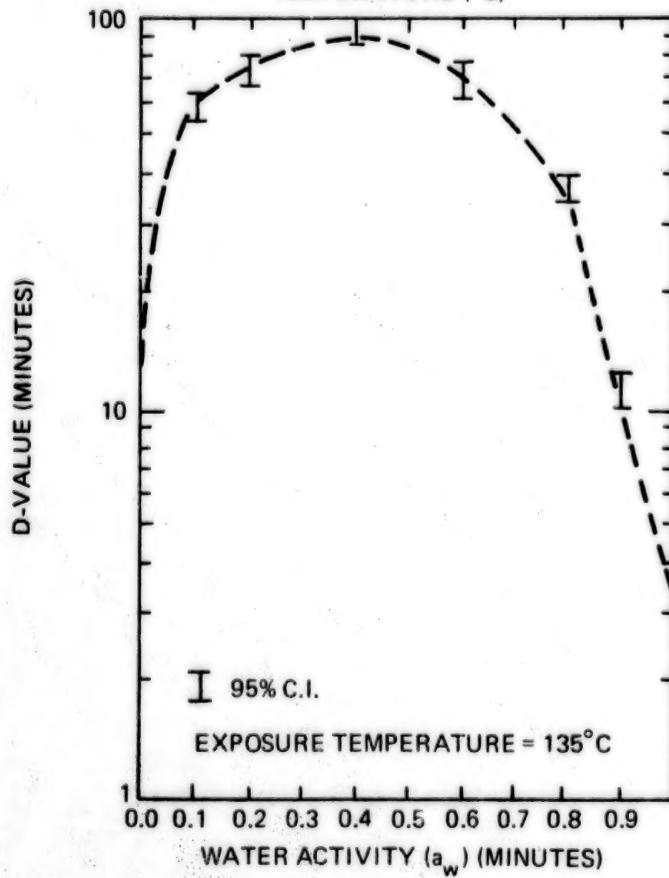


FIGURE 15. – Influence of water activity on dry-heat resistance of *Bacillus subtilis* var. *niger* spores encapsulated in Lucite (Angelotti et al., 1968a).

TABLE 1. - Dry Heat Resistance Values

Organism	D ₃₀₀ min.	z°F	Testing conditions	Source
<i>B. subtilis</i> (5230 or 15U)	5.2- 7.3	33	Hermetic cup-TDT can system five gasses, He, N ₂ , O ₂ , CO ₂ and air	Pheil <i>et al.</i> (1963)
<i>Clostridium sporogenes</i> (PA 3679)	6.0- 8.8	39-41	Hermetic cup-TDT can system three gasses, He, CO ₂ and air	Pheil <i>et al.</i> (1963)
<i>B. subtilis</i> (niger)	4.8	49	Filter paper strips in 150 x 16 mm screw cap tubes in aluminum block	Bruch <i>et al.</i> (1963)
<i>B. stearothermophilus</i> (FS 1518)	1.2	44		
<i>B. subtilis</i> (niger)	8.7-21.0	42	Added to or trapped in solids	Bruch <i>et al.</i> (1963)
<i>B. subtilis</i> (5230 or 15U)	7.0	42	Superheated steam in thermo- resistometer (300 to 350°F)	Pflug (1960)

temperature, to determine the relative sterilization effect.

Relative humidity (the ratio of actual water vapor pressure in a system and the unsaturated water vapor pressure at the same temperature) in gaseous systems corresponds to water activity in liquid systems. At equilibrium, the RH of the atmosphere surrounding the microbial cell is theoretically equal to the water availability inside the cell, which is called water activity (A_w), a definition suggested by Scott (1975). (The several measuring systems for water in gaseous systems are reviewed in Fisher *et al.* (1975) and equations and charts are included for conversion from one measurement system to another.)

Relative humidity is used in heat destruction studies to measure the amount of water in microorganisms, because the response of biological materials more nearly parallels vapor pressure than water content; and the vapor pressure is more easily measured and controlled than the water content inside the microbial cell, which cannot be measured directly. In reporting dry-heat test data in a microbial destruction rate test, it is suggested that the results be reported as a function of relative humidity, not water activity. When an RH value is reported, the temperature at which the RH was measured should be included; for example, 0.2 percent RH (110°C).

The actual movement of water to or from microorganisms on surface is determined by the RH surrounding the cell (Pflug, 1968). By increasing the humidity in air passing over microbial spores from near zero to 0.20, Silverman (1968) was able to consistently increase the D-value by a factor of a hundred and his data suggest that the maximum was not reached.

In the temperature range of 100°C to 123°C, spores of an intermediate moisture content (equilibrated at relative humidities between 0.1 and 0.6) are more resistant to the effects of heat (larger D-values) than spores of either greater or less moisture content. According to Marshall *et al.* (1963), who measured the moisture content of spores of six bacterial species equilibrated to various water levels at 25°C, the critical moisture content is between 5.5 and 12.4 percent of the dry weight of the spores depending on the species. (See table 2.)

In figure 15 is shown a curve of D-value versus spore water content; the details are from Angelotti *et al.* (1968a). The zero value is for an open system. It is added here along with the dotted line to indicate the probable D-value when spore water content approaches zero.

Brannen and Garst (1972) determined D-values at 105°C for relative humidities from 3×10^{-4} to

2×10^{-3} . They found that when $\log D$ was plotted vs. $\log RH$ between an RH of 1×10^{-3} and 1×10^{-2} , the data points formed a straight line. Jacobson and Pflug (1972) studied the dry-heat destruction rates of *Bacillus subtilis* var. *niger* spores at 90°C, 110°C, and 125°C at atmospheric water contents from 5 to 13,000 ppm, volume per volume (at 100°C this is an RH range from 7×10^{-6} to 7×10^{-3}). They found that in the range studies, the D-values decreased continuously as the RH calculated at test temperature decreased. When $\log D$ was plotted as a function of $\log RH$ at each test temperature, the data points formed straight lines. The lines for data at 90°C, 100°C, and 125°C were parallel. A decrease in the RH from 1×10^{-2} to 10^{-6} resulted in a 90% reduction in the D-value. The z-value was about 20°C and appeared to be constant over the temperature and RH ranges studied.

The large majority of data indicates that at temperatures in the range from 90°C to 135°C, the maximum dry heat D-value of heat resistant spores occurs at an RH between 0.2 and 0.5 with a z-value of about 21°C. The z-value appears to remain constant at about 21°C regardless of RH, when the RH is less than 0.5. The z-value for wet-heat, with an RH value of 1.00, varies from about 70°C to 12°C and is a function of both spores and substrate; therefore, the z-value decreases dramatically as the RH approaches 1.00.

Open and Closed Systems

The terms "open system" and "closed system" were suggested by Pflug (1968) to indicate the relative control of the heating environment on spore water loss or gain. In an open system, the spore water content will be determined by the environmental atmosphere surrounding the spore; while in a closed system, the spore water content is a function of conditions inside an enclosure and is not influenced by the heating environment.

In the closed system (fig. 16), water movement and water availability to the cells are restricted. The quantity of water that is available, or that can be transferred to or from the cell, is limited by the quantity of water initially present in the enclosure.

Two important parameters regarding cells in a closed system are initial water content and enclosure volume. The water concentration in the cell during

heating will be determined by the relative humidity of the atmosphere in the enclosure at the time of sealing the enclosure and the total volume of the enclosure. Changing either the quantity of water

TABLE 2. — *Moisture Content of Six Bacterial Species of Spores Equilibrated to Various Relative Humidities* (Data from Marshall, et al., 1963)

Relative Humidity	Range of Water Content (percent dry weight)
0.1	4.8– 8.2
0.2	5.5–10.2
0.4	7.3–12.4
0.6	9.5–16.0
0.8	12.1–25.5
0.9	38.5–57.0

TABLE 3. — *Thermal Death Times and D-Values (in Hours) at 248°F (120°C) for Spores of B. Subtilis var. niger Entrapped in Several Solids* (From Bruch, Koesterer and Bruch, 1963)

Compounds	Time to Sterilize	D-values*
Solid rocket propellant	24.0	2.5
Asbestos patching cement	20.0	2.1
Plaster of Paris	12.0	1.7
Glue-base marble patching plaster	30.0	4.0
Dental materials:		
Inlay investment A	4.5	0.6
Inlay investment B	30.0	3.2
Inlay die material	30.0	3.6
Bridge model material	15.0	1.6

*The D-values were calculated from levels of spore contamination found by assay of the solid materials. The weight of samples for a given solid was held constant and was in the range of 0.5 g to 1.5 g for all materials. Samples solidified around thermocouples showed that all solids reached temperature in 10 minutes.

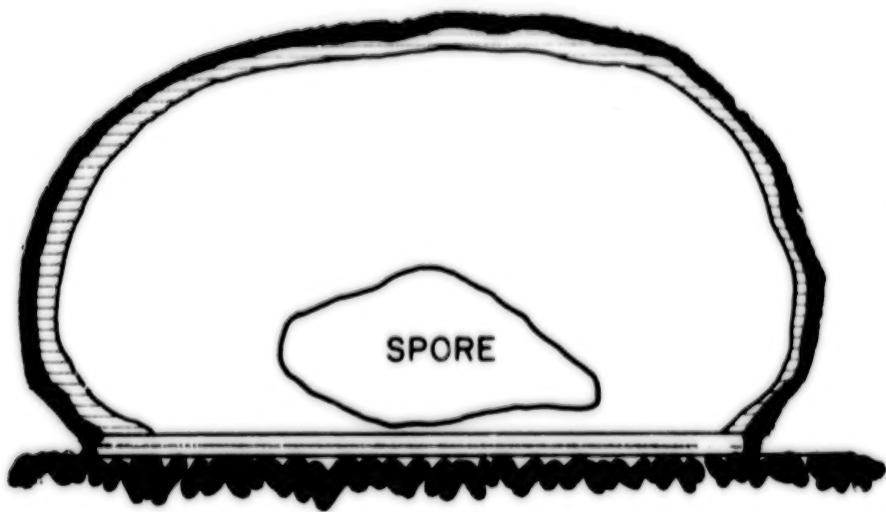


FIGURE 16. -- Diagrammatic concept of a microorganism in a closed system (Pflug, 1968).

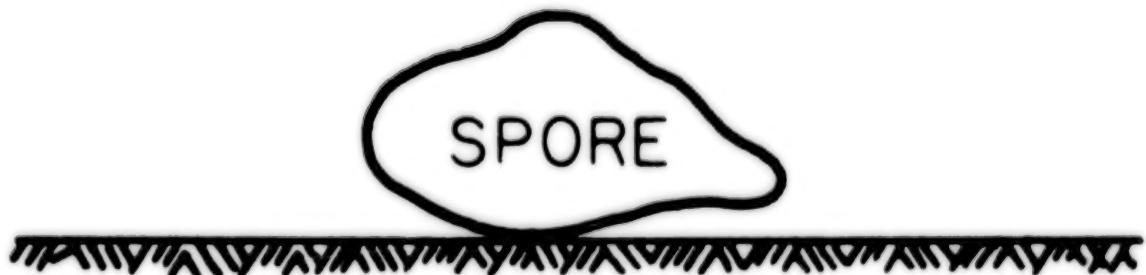


FIGURE 17. -- Diagrammatic concept of a microorganism in an open system (Pflug, 1968).

initially in the enclosed volume or the size of the enclosed volume itself will alter the relative humidity and, in turn, the water content in the spore during the heating cycle.

In the open system (fig. 17), water can be lost or gained by the heated cells almost without limit. In an infinite time the cells will be in equilibrium with the water condition of the environment. This defini-

tion places no restrictions on the rate of water transfer.

The open system describes the situation of microorganisms on nonporous surfaces such as laboratory glassware, glass and metallic slides, surgical instruments, and spacecraft.

Mated surfaces of space vehicles, or for that matter, of most hardware, are not usually hermetic; therefore, such surfaces are considered open systems. The rate of gas transfer between such surfaces and the sterilizing medium does affect the rate of spore destruction on the surfaces. This result is probably directly related to the rate of moisture loss from the cells. The rate of gas transfer between the surfaces and the rate of moisture transfer from the cells, are apparently affected by the physical characteristics of the joint or mating area; i.e., the pressure holding the joint together—including the deformation of the materials and the characteristics of their surfaces (refs. 16, 21, and 25).

The closed system describes the situation for microorganisms sealed within plastics; for example, in electronic parts and components.

Extending these concepts for spacecraft sterilization applications, NASA defined three categories of microbial contamination based on the capability of spores to gain or lose water (ref. 12):

- (1) Encapsulated Cells—Bacterial cells located in the interior of and entirely surrounded by solid material of a type essentially impervious to the transmission of water vapor
- (2) Surface Cells—Bacterial cells located on hardware in such a position that when the hardware is subjected to a dry-heat sterilization process, the cells are in intimate and continuous contact with the gas used for heating the hardware
- (3) Mated Surface Cells—Bacterial cells located or situated so that they are not classifiable as either surface cells or encapsulated cells.

Encapsulation of Spores in Crystals and Solids

Doyle and Ernst (1967) and Mullican and Hoffman (1968) both produced crystals in which *Bacillus subtilis* var. *niger* spores were encapsulated. Doyle and Ernst reported a nine-fold increase in the dry-heat resistance of spores encapsulated in calcium carbonate crystals compared to the nonencapsulated

controls. Mullican and Hoffman found that spores in glycine crystals were 5 to 24 times more resistant than spores in sodium chloride crystals were six times more resistant than the nonencapsulated spore controls. Encapsulating the spores increased the wet-heat resistance by 900 times.

The spores inside crystals were in a "closed system" compared to the spore controls which were in an "open system." The vapor tightness of the crystals is verified by the fact that ethylene oxide did not sterilize even after 48 hours.

The "wet heat" results suggest that wet-heat conditions existed on the outside of the crystal, but that inside the crystal (closed system) dry-heat conditions existed. The results of the encapsulation-in-crystal studies are similar to the results of Angelotti et al. (1968a) where the survival times were longer when spores were encapsulated in Lucite*. The very high, dry-heat resistance of encapsulated spores is probably due to the presence of the optimum amount of water in the spores to give near-maximum, dry-heat resistance.

Bruch et al. (1963) reported D-values for spores of *Bacillus subtilis* deposited on paper strips, sand, and glass tubes. The D-values were lowest when spores were deposited on the paper strips and highest when deposited on sand. They also studied the effect of the material in which the spores were entrapped.

Table 3 includes data from Bruch et al. (1963) for *Bacillus subtilis* var. *niger* spores entrapped in several solids. (The resistance of the suspension of *Bacillus subtilis* var. *niger* spores on a paper strip was 0.91 hour.) It would be of considerable value to know if these heat resistance values correlate with water activity of the particular material, or the water activity of the micro-area where the spores may be contacting the carrier. Are these differences a reflection of the hygroscopic characteristics of these carriers and as such, do they act as protectors of spore death in the manner that Greaves (1960) has described as a protective colloid and buffer system to maintain a minimum moisture level in freeze-dried spores?

Encapsulation of Spores in Soil or Soil Particles

When an object is in contact with people and ambient air, it will accumulate a microflora that can generally be divided into two groups: (1) Naked or

*Trademark of I. E. Du Pont de Nemours Co.

unprotected microbial cells of human, plant, or animal origin (these may be individual or clumps of naked cells that are part of a residue of food or other organic media that contaminate the objects to be sterilized); and (2) microbial cells associated with particles of dirt or dust that fall upon the object to be sterilized either from the air, or from the activity of persons or apparatus working near the objects to be sterilized (these organisms originate in the soil and are transported to the objects by air or humans).

Generally, the naked microbial cells are easily killed by a dry-heat sterilization treatment. In contrast, the microflora associated with soil particles are very difficult to kill.

The possible numbers and species of microflora in soil and the physical conditions of their location are almost unlimited. A soil sample can consist of a variety of species all produced under widely varying, unknown conditions; and the soil particles can vary in size and composition. The microorganisms can be located at any point on or within the soil particles. It is probable that due to cycles of soil wetting and drying, spores or vegetative cells can become completely encapsulated in soil particles. This process in itself could increase the dry-heat resistance of soil microflora by a factor of ten.

The normal microflora of soil is widely variable and includes both vegetative cells and spores. The 125°C microbial survivor curve in figure 18 is for 0.1 gram samples of a soil from Hennepin County, Minnesota. As is evident in the figure, this Minnesota soil contains a large fraction of very dry-heat resistant organisms—not only dry-heat resistant bacteria, but also large numbers of relatively dry-heat resistant actinomycetes.

Koesterer (1965a, b) reports on studies of the survival of organisms in a 0.1 gram sample of dry soil. Survivor curves for temperatures in the range of 120°C to 160°C are shown in figure 19. There is surprising similarity between the 125°C dry-heat survivor curves for Koesterer FG soil from upstate New York and the Minnesota soil.

Oxborrow et al. (1975) studied the relationship between particle size and viable microorganisms associated with airborne particles in two work areas. They found that 99 percent of the total number of particles present in both areas were less than one μm in size; only 0.01 percent of these particles contained viable microorganisms. At the other end of the particle size scale, it was found that less than 0.1 percent of the

total particles were greater than 5.4 μm in size, but 4.5 percent of these particles yielded viable microorganisms; therefore, the prevalence of viable microorganisms was 450 times greater in the larger particles. On the basis of this research, it was concluded that eliminating large particles in spacecraft assembly area will dramatically reduce the number of contaminating microorganisms.

Fallout particle load for clean room assembly of hardware was estimated by deRoos and Pflug (1971). They concluded that if the particle load is reduced, the microbial load is reduced; and if the microbial load is reduced, sterilization requirements are reduced.

Studies reported by Puleo and co-workers (1974) suggest that the relative number of resistant spores is small. They estimated that one spore between 10^3 and 10^5 will survive a dry-heat treatment of 30 hours at 113°C.

Nevertheless, soil contains some very dry-heat resistant species. If the surviving organisms of dry-heat tests are cultured in the laboratory, spore crops with high dry-heat resistance can be produced. Koesterer (1965), Bond, et al. (1970), and Campbell (1974) have all obtained dry-heat resistant isolates from soil and have cultured these resistant organisms to evaluate their dry-heat resistance. The resistance of these hardy organisms was found to be variable with D(125°C)-values ranging from 5 to 139 hours.

Although very resistant organisms are found in soil when many of the surviving organisms of dry-heat tests are cultured in the laboratory, the resulting spore crops have resistance levels of the order of *Bacillus subtilis* var. *niger*.

At the present time there is no survivor curve model for microorganisms in soil; therefore, the D-value cannot be used because it is a parameter of the straight-line, semilogarithmic survivor curve. The semilogarithmic survivor curve for microorganisms in soil has been described as being biphasic, since soil generally contains a large low-resistant population and a small high-resistant population of microorganisms. However, neither the low- nor high-resistant population is homogeneous.

A summary of the results of studies on the dry-heat resistance of microflora associated with soil particles suggests that:

1. The normal microflora of soil is widely variable and the semilogarithmic survivor curve for microorganisms in soil is nonlinear. A typical survivor curve for microorganisms in soil is shown in figure 18.

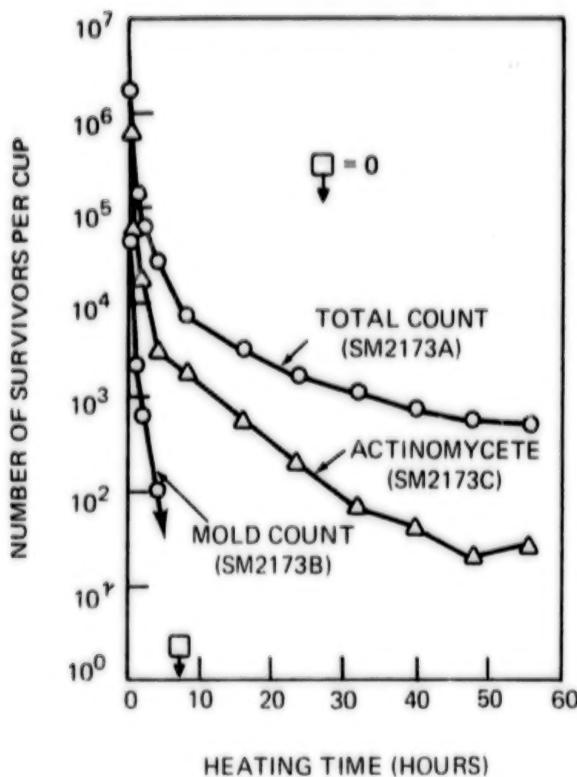


FIGURE 18. — Survival characteristics of bacterial, mold and actinomycete spores associated with dry Minnesota soil, heated at 125°C, 0.1 g soil per TDT cup.

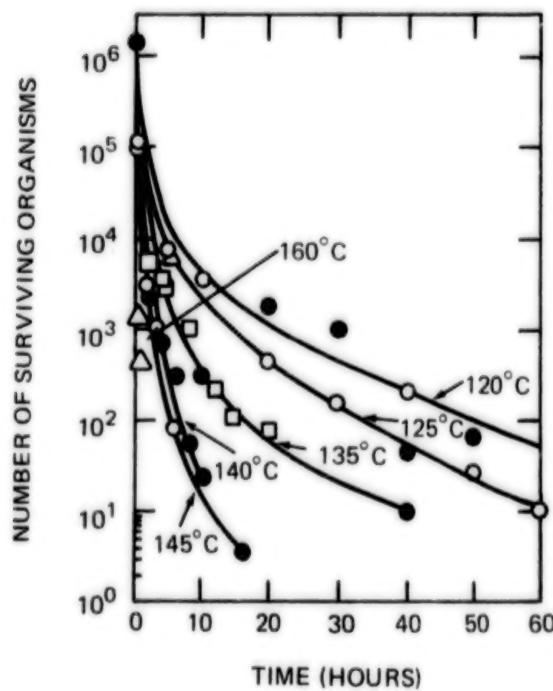


FIGURE 19. — Survivor curves for the indigenous mesophilic population in 0.1 g samples of dry FG soil to dry heat in the temperature range 120°C to 160°C (Koesterer, 1965a).

2. There are some very dry-heat resistant soil organisms. One such organism *Bacillus xerothermodurans* was isolated by Bond et al. (1973) and found to have a D(125°C)-value of 139 hours.
3. Moderately resistant organisms such as *Bacillus subtilis* can be very dry-heat resistant when encapsulated in particles.
4. The best estimate today is that in soil, one spore between 10^3 and 10^5 is very dry-heat resistant.
5. The fallout dirt or dust in a facility is part of the area dirt that has been carried into the facility by air or on clothing or equipment and has become airborne.
6. The role of the ambient relative humidity on the rate of spore destruction in soil heated in open systems has been studied, but the results are not conclusive. It is possible that the bound water in soil particles overshadows the effectiveness of the ambient humidity level on the spore destruction rate; for example, in many soil particles there are hydroscopic crystals that may cause organisms normally of low resistance to become more dry-heat resistant.

When objects are manufactured under ambient air conditions with adequate opportunity for soil particles to be deposited on the units to be sterilized, then the sterilization cycle will have to be based on the resistance of spores in or on the soil particles. If manufacturing is carried out under closely controlled conditions such as in a Class-100 clean room, then the number of soil particles per object could probably be reduced to the point where they are not the critical design criteria.

The naked microorganisms, grown in the objects to be sterilized or originating from the people working with the objects, will become the critical microbial load and the sterilization cycle will be based on the resistance of these naked organisms rather than the organisms associated with soil particles. Weather can have a major effect on dry-heat sterilization requirements. Humid conditions reduce and dry conditions increase soil particle movement. High wind velocities and dry soil will increase the number and size of soil particles and, therefore, the microbial load on objects to be sterilized.

In sterilizing an object contaminated by microorganisms associated with soil, we are dealing with a grossly heterogeneous microbial population. This

population will produce a survivor curve that will be similar in shape to the survivor curves in figures 18 and 19.

To gather data for the realistic design of sterilization processes, a test material must be selected that will have a microflora similar to that on the object to be sterilized. A survivor curve for this substrate must then be developed that can be used in the final sterilization design. Proceeding in the design, the microflora on the hardware to be sterilized must be evaluated, and quantitatively associated to the test substrate. All of the facilities for contamination control are used to minimize contamination on the material to be sterilized (deRoos and Pflug (1971) have evaluated some of the characteristics of particle containment in a preliminary way).

We would assume that the test survivor curve would cover a larger total microbial load than would be on the hardware; therefore, by using protective measures and cleaning procedures and by monitoring the relative contamination on the hardware, a parallel survivor curve could be established to reflect the contamination level. Locating the probability of survival level on this survivor curve will give the sterilization value F(+) to achieve this level of survival.

Gas Atmosphere

Heil et al. (1967) evaluated the effect of the gas atmosphere surrounding dry spores of *Bacillus subtilis* strain 5230 and *Clostridium sporogenes* strain PA 3679 over the temperature range, 121.1°C to 160°C (250°F to 320°F). In all cases they found that the effect of the different dry gases was small: *Bacillus subtilis* showed slightly more resistance in the inert gases, nitrogen and helium, than in oxygen, air, and carbon dioxide. The PA 3679 exhibited highest resistance in helium, least resistance in air, with resistance in carbon dioxide falling between the two but nearer the resistance in helium than air. The z-value was 18.3°C (33°F) for *Bacillus subtilis* in the five gases tested, 21.7°C (39°F) for PA 3679 in helium and carbon dioxide, and 22.8°C (41°F) in air. Bruch (1966) included data from Koesterer (1962) that showed microorganisms to have the greatest heat resistance in an air atmosphere with decreasing resistance in a helium atmosphere and in a vacuum. The reported relative D-value effect of air and helium was approximately 2 to 1, whereas Heil et al. (1967) found a relative air to helium D-value effect of 5 to 6.

These differences may be due to the type of experimental system used. Pheil et al. (1967) used a closed system while Koesterer (1962) used an open system.

Simko et al. (1971) determined the dry-heat resistance of *Bacillus subtilis* var. *niger* spores on mated surfaces in an air, nitrogen, and helium atmosphere. They found no difference in the resistance of the spores in the two dry gases, nitrogen and helium. The spores had a significantly greater resistance in air. Since ambient air was used, its higher water content was probably responsible for the higher spore resistance in air.

Davis et al. (1963) found that the survival of dry spores at 60°C and high vacuum was consistently lower than for dry spores held at atmospheric pressure for the same length of time; whereas at 25°C there was little difference in survival at atmospheric pressure and a high vacuum. Survival at a temperature of 88°C growth (upon subculturing) was considerably less than at 60°C and 100°C; in general there was no growth (upon subculturing) after four to five days at high vacuum. The relative vapor pressure of water at these temperatures was: 25°C, 23.756 mm of mercury; 60°C, 149.38 mm; 88°C, 487.1 mm; and at 100°C, 760 mm. Comparison of the vapor pressure values (a measure of the rate of dehydration when measured against a hard vacuum) pointed out that the drying rate was more than 6 times faster at 60°C than at 25°C; more than 3 times faster at 88°C than at 60°C; and 50 percent faster at 100°C than at 88°C. Destruction rates seemed to approximately parallel drying rates. These data appear to parallel data of Marshall et al. (1963) and Murrell and Scott (1957) who reported that spore viability decreased when spores were exposed to a near-zero water activity or, in other words, were subjected to a severe drying stress.

Bruch et al. (1963) observed, when discussing their data and the data of David et al. (1963), that "Clearly, the presence or lack of gaseous environment surrounding microbial spores during dry-heat sterilization influences the rate of spore destruction. The composition and stability of the gaseous atmosphere also may be important and may be the factor responsible for the high dry-heat resistance of spore samples on sand and soil samples."

When microbial spores are heated in a superheated steam atmosphere, they are subjected to dry-heat conditions. At equilibrium there will be no liquid water present and the relative humidity will be less than 1.00. It is perhaps confusing, but true, that we

can have a 100% water vapor atmosphere and still have a relative humidity below 100% and dry-heat conditions. In a 100% superheated steam atmosphere the relative humidity is determined by the pressure and temperature of the system. The saturated vapor pressure is determined by the temperature of the system. The vapor pressure will be the actual pressure of the system. Reducing the pressure while holding temperature constant will reduce the relative humidity.

DESIGN FOR SPACECRAFT STERILIZATION

In the preceding sections of this chapter, we have discussed the factors affecting the resistance of both natural and laboratory microorganisms to dry-heat sterilization and how the resulting data were used to develop a concept for the design of spacecraft sterilization processes. What follows is a brief summation of the conclusions reached and the resulting design concept. The final section of this chapter will discuss in length those particularly hardy microorganisms which are not incorporated into this design.

As was mentioned earlier, the spores of *B. subtilis* var. *niger* are used as the test or reference organism in studies of dry-heat resistance. In studying the influence of water on dry-heat resistance, investigators found that the uptake and loss of water vapor by *B. subtilis* var. *niger* spores is very fast and reaches equilibrium in approximately 20 minutes (ref. 16). The amount of water available to a spore both before and during heat exposure, can significantly affect its thermal resistance to the point that it can be an overriding factor. At the lethal temperatures studied, microorganisms are most resistant when the relative humidity of the environment is in the range of 30 to 50 percent. At both lower and higher relative humidities, their resistance is decreased. In some situations, the amount of water is more important than moderate temperature changes in controlling microbial thermal inactivation rates (ref. 16). In general, if objects to be dry-heat sterilized are held in a low-humidity environment prior to the sterilization treatment, the probability of achieving sterilization is increased.

Based on this information and data accumulated by many investigators, NASA developed a concept for sterilization. For example, prior to the final assembly of the Viking spacecrafst, all components were subjected to a heat sterilization test sufficiently severe to destroy all buried or encapsulated organisms. Then, the terminal sterilization process

(where the final assembled spacecraft was treated as an entity) had to destroy only the organisms which were deposited on the exposed and mated surfaces during the assembly operations. NASA decided not to control the humidity during assembly of spacecrafst, because of the practical problem of electrostatic charge buildups and human constraints associated with such presterilization humidity control.

However, to achieve a high probability of sterilizing the organisms on exposed and mated surfaces, a low relative humidity was used during the terminal sterilization. NASA used a dry, inert gaseous environment for the final sterilization process to take maximum advantage of the benefits of low humidity.

Design Standards

NASA developed the following constraints (ref. 12) to be used in establishing dry-heat sterilization processes for spacecraft. The thermal resistance characteristics of the bacterial population that are used to calculate the death of bacteria shall not be less than the following, providing that the gas used in the thermal heat cycle be as dry as physically possible throughout the process, and that continuous monitoring be conducted to measure the moisture content during the cycle:

- D-value at 125°C "dry heat" for "surface cells" = $D_{125} = 0.5$ hour
- D-value at 125°C for "encapsulated cells" = $D_{125} = 5.0$ hours
- D-value at 125°C "dry heat" for "mated surface cells" = $D_{125} = 1.0$ hour
- z-value = 21°C

With this understanding of dry-heat sterilization and the technology of sterilizable parts and materials, NASA first demonstrated the feasibility of dry-heat sterilization of spacecraft by using a development vehicle with the acronym of CSAD.

This design was also the basis for sterilizing Vikings I and II, and it is now generally agreed that the Vikings were "successfully" sterilized—the real proof of the sterilization technique.

However, this technique was based on the microbial assay of mesophilic aerobic microorganisms (ref. 26); because early investigations found almost no obligate anaerobes in the spacecraft environments (refs. 27 and 28). On the other hand, attempts were not made to isolate psychrophiles, organisms that grow at low temperatures, and omnitherms that

grow over a broad temperature range. Contamination by them will be significant in future flights to the cold planets of Jupiter and Saturn and also in the food industry. The following section will discuss their hardy characteristics in an effort to develop a sterilization design.

PSYCHROPHILES AND OMNITHERMS

Psychrophiles

The significance of psychrophilic organisms was indirectly reported by Hawrylewicz et al. in 1967 (ref. 29), when they postulated that due to water availability under the low atmospheric pressure of Mars, growth of microorganisms would necessarily be confined to temperatures below 8°C—a temperature conducive to the growth of psychrophiles but not mesophiles.

While it is probable that mesophilic organisms do not threaten to contaminate Mars because of their inability to grow at the low Martian temperatures; psychrophiles are a probable threat to Jupiter, Saturn, and their satellites, because of their reported survivability at the recorded low temperatures of these bodies. Therefore, the microbial assay of psychrophiles on future interplanetary spacecraft is essential in meeting the standards set by the international agreement on planetary contamination (ref. 28).

This discussion is not intended to be a detailed treatise on psychrophilic microorganisms, but rather to draw attention to their importance in planetary research and quarantine and the need to develop a design for sterilization. Such a design is not only crucial to the space industry, but to the food and other nonspace industries as well.

Investigations of organisms growing at low temperatures have for years been associated primarily with food industries because of the significance of these organisms in food spoilage. Their ability to grow at low temperatures was recognized by early scientists such as Forster in 1887 (ref. 30), who reported growth at 0°C, and Ekelof (ref. 31) in 1901, who reported the presence of psychrophiles in his studies in the Antarctic.

Since those early investigations, numerous articles have been published on the isolation of psychrophiles, and these investigations have been reviewed by several authors (refs. 32-36). It was in 1902 that Schmidt-Nielsen first used the term "psychophile" (ref. 37).

He simply stated that psychrophiles were not only able to survive, but could also multiply at 0°C. Since then, there has been a great deal of controversy over the terminology associated with this group of organisms. Numerous and contradictory definitions of "psychrophile" (refs. 38 and 39) were based on optimum growth temperature, maximum temperature for growth, time required for good growth (e.g., visible growth in 7-10 days at 0°C), or various combinations of these criteria.

Due to confusion in the literature on definitions of the term, various investigators attempted to alleviate the problem by proposing new terms (refs. 40 and 41). In 1954, Hucker (ref. 42) proposed the terms "obligate" and "facultative" in describing this group, and in 1960 Eddy (ref. 43) proposed the term "psychrotrophic" to include all organisms growing at a low optimum temperature. For an excellent discussion of this history of terminology, the recent review of Morita (ref. 44) should be consulted. He attempts to clarify these investigations and defines psychrophiles as those organisms having optimum growth at about 15°C or lower, maximum growth at about 20°C, and minimal growth temperature at 0°C or lower. Although this definition may be challenged by future investigators, Morita's presentation is quite reasonable, and his suggestions would eliminate a great deal of confusion in this field.

Of more interest than the terminology is the physiology of psychrophiles, which are non-mesophilic. In other words, they possess unique characteristics which enable them to grow at lower temperatures than mesophiles. The majority of microbial investigations have been concerned with mesophilic organisms because of their immediate impact on our lives. The psychrophiles also have tremendous impact on us, but it is apparently more subtle. Their greatest impact is felt in food spoilage, but they probably affect us in many more ways and much research is needed.

For example, psychrophiles are significant in their suspect role in planetary contamination. Recent work by Foster and Winans (ref. 28) clearly demonstrated the existence of psychrophiles from areas associated with the Viking spacecraft and pointed out that these organisms are excluded from present microbial monitoring procedures. One of their significant findings was isolation of psychrophilic *Bacillus* sp. from Cape Canaveral. This will be discussed later in this chapter.

Several authors have investigated the selection of bacterial populations imposed by the environmental conditions of deep space (refs. 45 and 46). They have demonstrated that space environments may select for organisms more capable of growing in a planetary environment. Similar investigations have been performed on psychrophilic populations and in 1971 Harder and Veldkamp (ref. 47) demonstrated that at low temperatures psychrophiles will outgrow psychrotrophs in continuous culture.

These and similar laboratory findings suggest a continuous selection for bacteria whose activity is greatest near the environmental temperature. Because of the extremely low temperature of deep space, the measured low temperature of the Martian environment, and the low temperature at which the Viking Lander Biological Instrument is maintained, it could be postulated that all of these low-temperature conditions would select for psychrophilic populations whose characteristics might predispose them to grow in the environmental conditions of Mars. Similar arguments could be made for selection of these highly adaptable organisms during missions to other planets that have different environments.

A recent paper by Olsen (ref. 48) demonstrates that mesophilic organisms can be genetically altered by transduction to become psychrotrophic. (Whereas psychrophiles grow at 20°C or lower, psychrotrophs grow at low temperatures above 20°C.) Because of the low temperatures imposed on an interplanetary spacecraft and the low temperatures of the planet, these altered mesophiles could begin growing and result in a significant problem for planetary quarantine. Plans are under way to investigate this in more detail.

Reported time for the generation of psychrophiles has been quite varied. Times ranging from 30 hours at 0°C to 6 hours at -5°C have been reported and several isolates have been reported to grow at -5.5°C or lower (ref. 44). Lower growth temperatures for psychrophiles have been reported in the literature, but many of the older reports are thought to have used unreliable refrigeration units. If one considers that there is little chance for life to exist on the planets now under investigation, the importance of generation times will be diminished. Without biological competition, organisms capable of contaminating a planet could do so even with prolonged generation times.

Much work has been done on the transport of substrates through the cell membrane at different temperatures (ref. 49). This work indicates that the uptake of substrates is decreased as the temperature is decreased; and that the proportion of unsaturated fatty acids in the membrane lipids increases as the temperature of growth is lowered. These results indicate that the mechanism of psychrophily may be associated with cell membrane fatty acids. If there is such a correlation between unsaturated fatty acid content, substrate uptake, and psychrophily, these investigations should be extended in order to extrapolate the results of this correlation to the proposed temperatures of the planets. The planetary temperatures might be so low as to make substrate uptake impossible, thus negating the possibility of psychrophilic growth in planetary environments.

Additional factors affecting the growth of psychrophiles or psychrotrophs are salinity and pressure. It appears that growth rate based on salt concentration versus temperature produces a dual effect. Summarized, the results show that a decrease in the maximal growth temperature of psychrophiles occurs at both high and low salt concentrations (ref. 50). Because very little moisture is expected to be on the planets, any that is there will probably be saturated with minerals. This will result in a high salt concentration, which will lower the maximum growth temperatures of the potentially contaminating organisms. It has also been demonstrated that increased salinity provides better survival in a freeze-thaw environment (ref. 51); because of the low temperature of the planets, these facts might increase the probability of planetary contamination by these organisms.

Other studies indicate that pressure has a negligible effect on the growth of microorganisms (ref. 52). Organisms can grow over a broad range of pressure changes, probably broader than will be experienced in investigating the planets. Although it is true that pressure will affect the physiology of the cell, and pressure differences of 100-300 atmospheres may decrease growth rate, it is unlikely that the pressure differences found between earth and the planets will be sufficient to prevent cell growth.

Numerous investigators list the different types of organisms that are generally considered psychrophilic (refs. 35, 36, and 44). In almost all cases, the predominant types are gram-negative rods. Gram-positive cocci, yeasts, and molds, and nonsporeforming gram-positive rods have also been described. All of these organisms are significant and the importance of each

type is determined simply by the subject. In the case of potential planetary contamination, sporeforming psychrophiles are most pertinent. This belief is based on the fact that prior to launch, the Viking Landers were decontaminated with dry heat (which does not significantly affect the hardy sporeformers), and because the environment of deep space is more detrimental to the nonsporeformers than the sporeformers.

In the early studies of psychrophiles, little attention was given to sporeformers. However, in the 1960's, attention was turned to these organisms because of the spoilage of pasteurized milk due to psychrophilic sporeformers. In 1965, Larkin and Stokes made a deliberate attempt to isolate psychrophilic *Bacillus* sp., and they clearly established that several isolates grew at 0°C, and one isolate grew at -4.5°C (ref. 53). Similar results have since been demonstrated by others. In 1964, Sinclair and Stokes reported the isolation of psychrophilic *Clostridium* sp. (ref. 54).

In these last two studies, maximum growth temperature of most isolates was above 20°C; therefore, these should probably be called psychrotrophs. Other investigators (ref. 28) also reported isolation of psychrophilic *Bacillus* sp., but because of the intent of their investigation to find organisms growing below 32°C, most of their isolates should also be called psychrotrophs.

Because of the threat of these isolates to contaminate Mars via the Viking Landers, it was deemed imperative to answer two questions: (1) would psychrotrophic *Bacillus* sp. reproduce in a simulated Martian environment; and (2) what is the dry-heat resistance of these isolates? Both questions have been investigated, and results, documented in semiannual reports (refs. 55-59), showed psychrotrophic *Bacillus* sp. to be capable of growing in a simulated Martian environment. Investigations for dry-heat resistance showed D-values ranging from 7.54 minutes to 122.45 minutes at 110°C and D₁₂₅-values ranging from less than a minute to ten minutes for 15 isolates tested (ref. 60). These studies demonstrated that the dry-heat cycle used on the Viking Landers should have easily killed these psychrotrophic sporeformers (Winans, Pflug, Foster – in press).

Numerous investigators over the last fifteen years have experimented with simulated Martian environments; but again, recovery of organisms surviving or growing in such an environment was usually performed at 32°C. Using new data on the Martian environment, investigators performed similar experiments with

recovery of organisms at 3°C to 7°C (refs. 55 and 56). Their results demonstrated that samples recovered at this low temperature showed rapid growth if moisture and nutrients were available. Results of similar studies using the slide-culture technique showed the formation of microcolonies in as little as 72 hours (ref. 57). All of these investigations employed a diurnal freeze-thaw cycle of -65°C for 16 hours to 20°C for 8 hours. The results demonstrated that this cycle did not adversely affect some populations which grew very well.

In a recent publication, Meyer et al. (ref. 61) investigated the effect of the freeze-thaw cycle alone (other Martian parameters were not investigated) on a mesophilic yeast and a psychrophilic yeast. Their results demonstrated that the growth of psychrophilic yeast is more retarded by the freeze-thaw cycle than is the mesophilic yeast; however, due to the use of only one organism and the exclusion of other Martian environmental parameters, their results do not necessarily indicate that the freeze-thaw cycle alone is sufficient to prevent contamination of Mars by psychrophiles.

Because of the interest in low-temperature incubation, investigators performed several studies of cooperative research with the PQ Lab at Cape Canaveral. Of five heat-resistant (hardy) sporeformers examined, three grew very well in a simulated Martian environment when moisture and nutrients were added (ref. 58). In another experiment, heated teflon ribbons from Cape Canaveral were subjected to a simulated Martian environment and held at 15°C (temperature of the Viking Lander Biology Instrument). Recovery of hardy organisms was decreased slightly, but they were isolated, indicating that the low temperature and the Martian environment could not be relied upon to prevent contamination of Mars by hardy organisms (ref. 58). In examining the temperature ranges of some of the hardy organisms, it was found that of twenty-four isolates examined, four grew very well at 3°C (these were originally recovered at 32°C), and six others grew well at 15°C. Of the four growing at 3°C, the one which grew best (M4-6) is still under intensive investigation because of its pronounced heat resistance. The findings strongly indicate that some of these heat-resistant organisms definitely possess the ability to grow at low temperatures (ref. 58). These and previous results also indicate that although psychrophilic sporeformers may not be extremely heat-resistant, some of the heat-resistant sporeformers will grow at low temperatures.

It has been demonstrated that the majority of psychrophiles studied thus far possess thermolabile enzymes or enzyme systems (ref. 44) and begin losing their activity above 20°C. If this is true, the psychrophiles would probably be of little concern in contamination of interplanetary spacecraft because of constant exposure to temperatures greater than 20°C. On the other hand, psychrophiles have been reported from samples previously exposed to temperatures higher than 20°C. Also, the importance of psychrotrophs not growing at 32°C has been described above, and they have been shown to be present in interplanetary spacecraft environments (ref. 28). They do not appear to possess the sensitive thermolabile enzymes demonstrated in some psychrophiles. If standard procedures were developed by NASA for the isolation of low-temperature organisms, it would be advisable to establish a dual protocol. The procedure for examining a sample for psychrotrophic organisms should not require undue refrigeration of the sample. However, a method to isolate psychrophilic organisms should include constant refrigeration of the sample, use of chilled media and instruments, and similar measures to prevent inactivation of psychrophilic thermolabile enzymes.

Although this discussion has been concerned only with low-temperature organisms and primarily with Mars; many investigators are recognizing the need to investigate organisms of special interest to contamination of the other planets as well. For example, Dimmick et al. (ref. 62) looked for organisms growing at high pH, and in aerosols—parameters of special significance to Jupiter. In other studies on calculating the probability of contamination (Pc) of Mars (ref. 63), Judd points out the need to estimate a sample's population of organisms capable of growth in ice in order to satisfactorily calculate Pc. Such information should be of value to others involved in mathematical modeling or statistical analyses, either related to planetary contamination, or to contamination problems in nonspace industries.

Omnitherms

Thus far, all omnitherms isolated have been sporeformers, many of which have the ability to grow aerobically and anaerobically over a broad temperature range. Omnitherms are important because they possess the characteristics to adapt to wide variations in the uncertain environmental

conditions of the planets. These organisms are presently being investigated in detail, especially their resistance, physiology at different temperatures, growth requirements, and similar characteristics of importance in determining their significance to planetary contamination.

Due to the isolation of sporeforming psychrophiles from Cape Canaveral soils, an investigation was performed to describe the bacterial population of the soil based upon incubation temperature and oxygen requirements. In this experiment, heat-shocked and nonheat-shocked soil samples were used. These investigations began during the summer of 1975 and preliminary results are presented in a semiannual progress report (ref. 59). (The isolates described below are currently under intensive investigation, and papers on preliminary results are now being prepared for publication.)

Fresh soil samples were obtained from Cape Canaveral and included samples from the Vehicle Assembly Building, Spacecraft Assembly and Encapsulation Facility (1 and 2), launch complex 41, and the fuel storage area. Equal portions of these were mixed to yield the Cape Canaveral soil used in this analysis and replicate samples were processed as shown in figure 20. After counts were performed, individual colonies were transferred from plates containing 30 to 300 colonies to four Trypticase Soy Agar (TSA) slants and incubated as shown in the figure. The objective of this step was simply to determine which of the low- and high-temperature isolates would not grow at 32°C. The extreme temperatures (55°C for the 3°C isolates and vice versa) were included to allow for all possible combinations of growth. It was felt that with hundreds of colonies being transferred, one additional TSA slant might provide additional information.

Counts for this initial investigation are shown in table 4 and are consistent with previous investigations on psychophilic organisms. Surprisingly, when growth from the TSA slants was recorded, it was noted that some of the isolates grew at 3°C, 32°C, and 55°C. Table 5 shows the percentages of the various groups of isolates and the ones in question are referred to as "omnitherms." The word "eurythermal," suggested to describe organisms having a broad temperature range (ref. 40), might be appropriate; but it was decided not to use this term at present because of confusion with "eurithermophile." Other terms such as "omniphile" and "psychrotherm"

have been suggested, but for this discussion "omni-therm" will be used.

TABLE 4. — *Counts of Mixed Cape Canaveral Soil Incubated Under Various Conditions (Per Gram of Soil)* (From ref. 59)

Treatment	Atmosphere	Temperature	Count (Average of 4 Plates)
Nonheat- Shocked	Aerobic	3°C	2.7 x 10 ⁵
		32°C	6.6 x 10 ⁶
		55°C	4.0 x 10 ⁵
	Anaerobic	3°C	3.9 x 10 ³
		32°C	5.1 x 10 ⁵
		55°C	6.8 x 10 ²
Heat- Shocked	Aerobic	3°C	2.1 x 10 ³
		32°C	8.9 x 10 ⁵
		55°C	2.2 x 10 ⁵
	Anaerobic	3°C	<1.0 x 10 ²
		32°C	1.8 x 10 ⁵
		55°C	2.4 x 10 ²

One very important aspect of table 5, which should be mentioned, is that the standard isolation procedures employed by NASA do account for the majority of the populations. In the case of the non-heat-shocked samples, 97.5 percent of the isolates grew at 32°C and 92.4 percent grew aerobically. With the heat-shocked samples, 95.3 percent grew at 32°C and 97.8 percent grew aerobically; however, the table also shows a large percentage of isolates which did not grow on subculture, and these could influence results. Improved procedures for transferring this many individual colonies are yielding a transfer rate of approximately 95 percent.

Thirty-four colonies of omnitherms, exhibiting the ability to grow from at least 3°C to 55°C, were isolated. The obvious explanation for this phenomenon appears to be mixed cultures in the isolated colonies; therefore, all colonies were streaked on TSA to determine if the cultures were pure. Individual, well-isolated colonies were transferred to TSA slants and Trypticase Soy Broth (TSB) and incubated again at

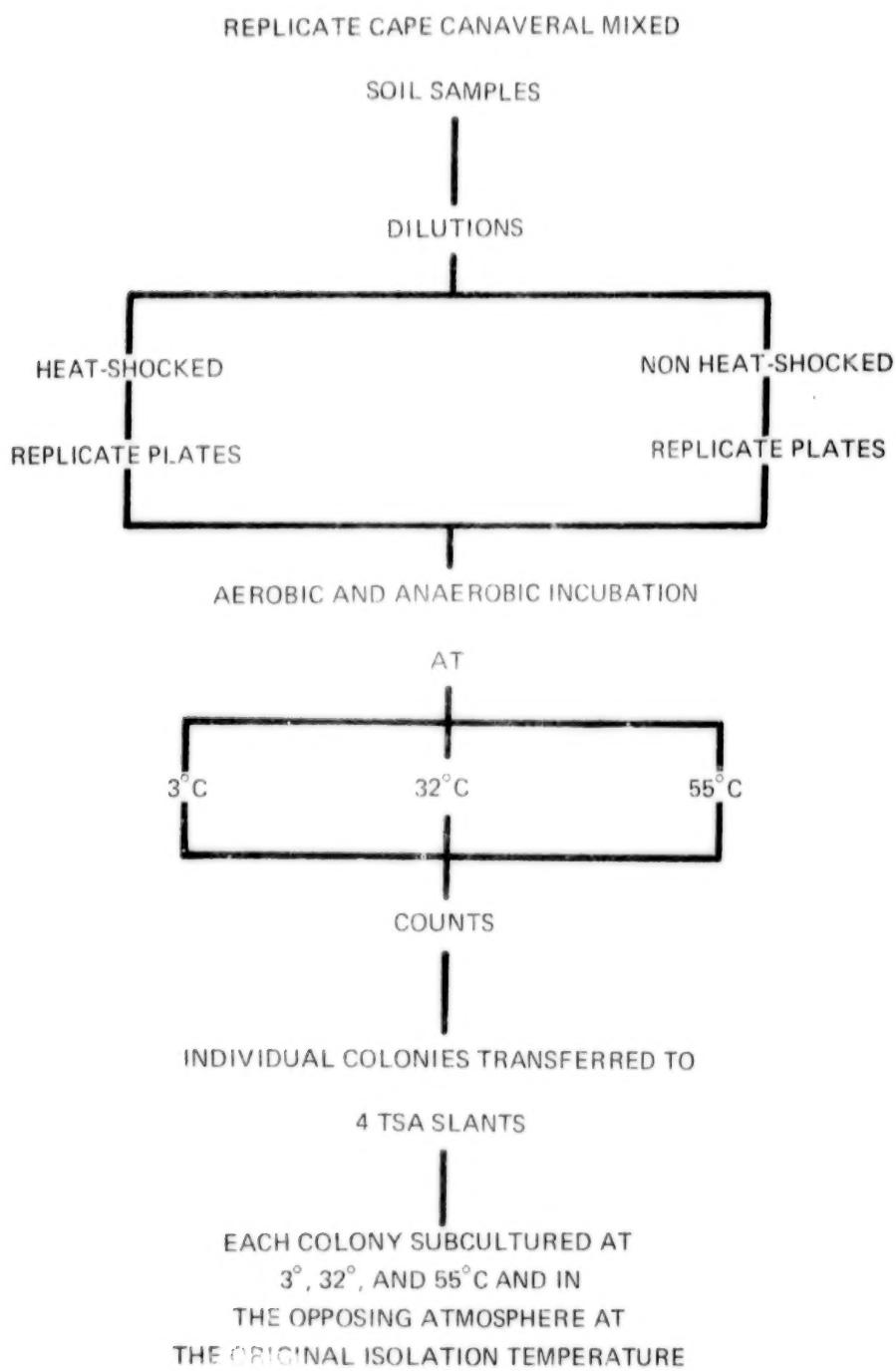


FIGURE 20. – Schematic demonstrating procedures for population profile study of mixed Cape Canaveral soil samples (from ref. 59).

TABLE 5. — *Population Distribution of Various Types of Organisms Isolated From Heat-Shocked Cape Canaveral Soil Samples (Given in Percent)*

Organism Type	Percent of Total Population	Nonheat-Shocked		
		Aerobes	Facultative	Anaerobes
Psychrophiles	1.4	0.6	0.3	0.6
Facultative Psychrophiles	32.7	2.4	26.7	3.5
Mesophiles	41.3	12.5	26.0	2.8
Facultative Thermophiles	8.2	7.2	0.7	0.3
Thermophiles	1.1	0.6	0.4	0.1
Omnitherms	3.4	0.6	2.5	0.3
No Growth on Subculture	11.9	—	—	—
Heat-Shocked				
Organism Type	Percent of Total Population	Heat-Shocked		
		Aerobes	Facultative	Anaerobes
Psychrophiles	1.0	0.6	0.4	0.0
Facultative Psychrophiles	8.4	6.2	2.2	0.0
Mesophiles	43.7	20.0	23.2	0.6
Facultative Thermophiles	10.8	6.9	3.3	0.6
Thermophiles	3.7	2.4	0.4	0.8
Omnitherms	2.0	0.6	1.2	0.2
No Growth on Subculture	30.4	—	—	—

the three temperatures and the resulting growth was stained. This was repeated no less than six times by different investigators. Individual colonies were examined for their omnithermal characteristics. During subsequent incubation, some of the isolates lost their ability to grow at all the temperatures and some of the cultures did not grow on subculture; but presently there are at least 29 omnithermal isolates. Although these grow at least from 3°C to 55°C, investigations on the cardinal temperatures of these isolates are not complete, and it is anticipated that the temperature ranges of most of these will be even broader than reported here. In order to better demonstrate that these isolates were actually growing over this broad temperature range, isolated colonies grown at 3°C were transferred to TSA and immediately incubated at 55°C and vice versa. Apparently, these organisms do possess the ability to be transferred from one extreme to the other and grow quite well.

Growth curves were plotted in one of the first experiments performed on these isolates. Five isolates were examined in liquid culture with turbidimetric measurements being made periodically. Of the five, one failed to grow at 3°C, three grew sparsely at 3°C, and the last grew very well at 3°C. They all grew well at 32°C and 55°C and the results of the latter organisms are shown in figure 21. As exemplified in this figure, the optimum temperature for the majority of these isolates is around 32°C. Many of them show

visible growth at 55°C only after 3 to 4 days, and most show growth at 3°C in 10 to 14 days.

Tentative identification of 29 of these isolates at 32°C have been made. Procedures used for identification are from Ruth Gordon's *The Genus Bacillus* (ref. 64). In performing these biochemical tests and limited temperature studies, it was determined that in at least four cases, cultures thought to be mixed were identified as the same species. It is thought that these are simply colonial variants of the same organisms; therefore, the number of mixed cultures appears to be less than originally reported.

One of the criteria used in identification of *Bacillus* sp. is the growth of the organism in anaerobic agar. Most of these isolates showed no growth in the anaerobic agar, but experiments were performed with the Brewer Anaerobe Jar and Gas-Pak system to determine anaerobic growth at 3°C, 32°C, and 55°C. The results showed that most of the isolates were facultative in their oxygen requirements. Some of them grew under anaerobic conditions at one temperature and not another, whereas some grew anaerobically at all three temperatures.

Because omnitherms have been isolated only recently, relatively little data is available at present; and it is too early to evaluate their role in planetary contamination. It appears, however, that the study of omnitherms will be advantageous to space research and to nonspace industries.

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HEAT STERILIZATION

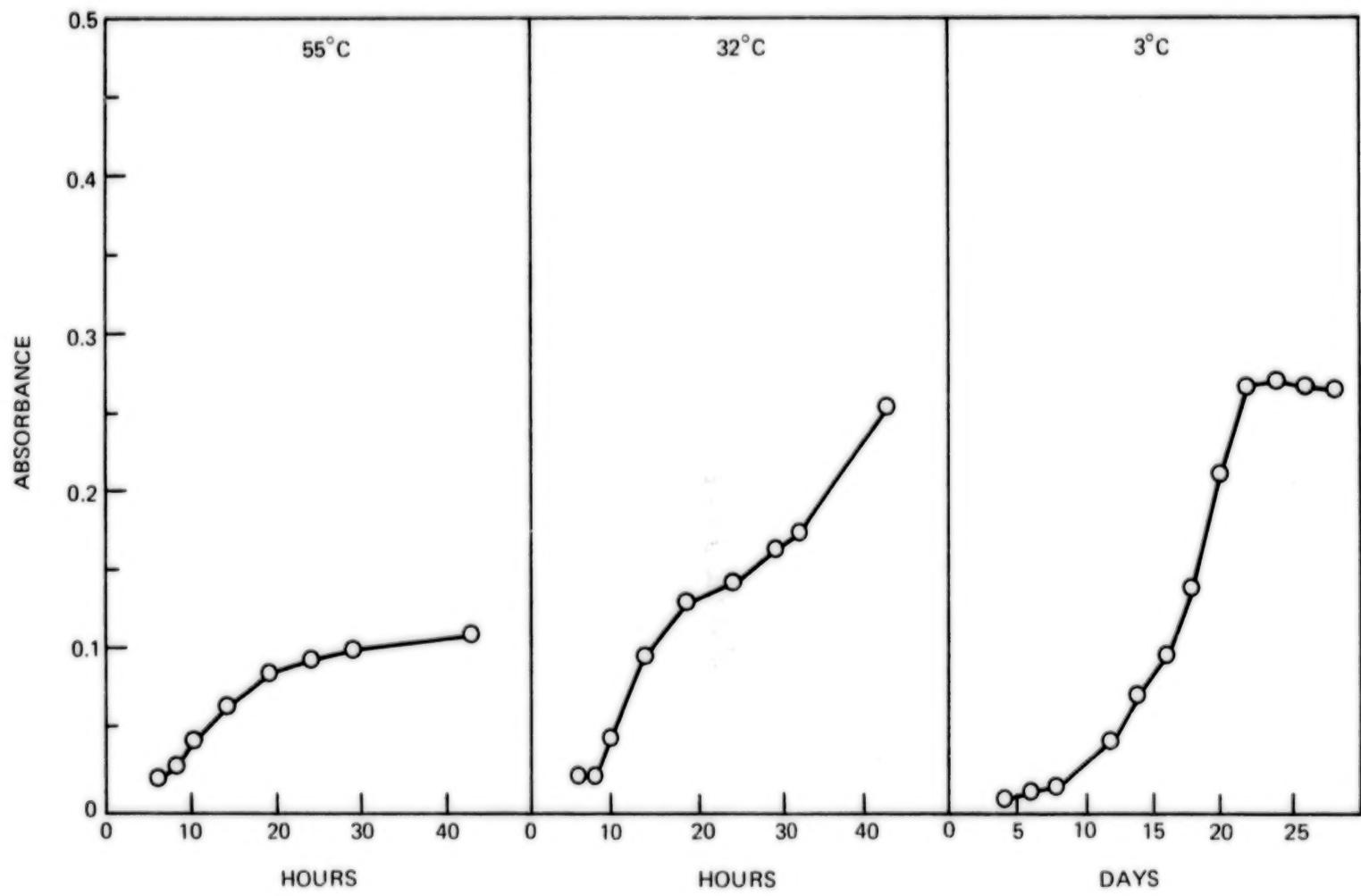


FIGURE 21. - Response of KSC soil isolate G-38B at three different temperatures.

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CHAPTER 3

Radiation Sterilization

"They froze it in a freezer that was cold as Banished Hope"

INTRODUCTION

Radiation sterilization, commonly called "cold sterilization," uses ionizing radiation (X-rays, beta rays, gamma rays), thermoradiation, or ultraviolet radiation to destroy the reproductive ability of an organism. Most microorganisms are sensitive to radiation; however, the degree of their response varies depending on the organism, type of radiation, exposure conditions, and presence of interfering or protective factors.

Radiation sterilization has a large number of applications. Ionizing radiation is used to sterilize blood products, disposable surgical articles, and drugs. It is also employed in vaccine production in the United States and for foodstuffs in Europe. (Federal regulations in the United States require that radiation be considered an additive when used for foodstuffs, and manufacturers must now prove that it can be employed safely.)

Thermoradiation, a relatively recent advancement combining a heat treatment with ionizing radiation, is so new that many applications are just now being developed. This process enables industrial manufacturers to sterilize delicate products and still maintain the utmost in quality.

Ultraviolet radiation is being applied to decontaminate air systems, surfaces, containers, and rooms. Ultraviolet radiation is not discussed in this chapter, since the state-of-the-art has not yet been significantly advanced from NASA-supported research.

The advantages and disadvantages of ionizing radiation sterilization are:

Advantages

- It can be used on many materials that are sensitive to heat and moisture.

Advantages - Continued

- There is no lag time before full exposure to its effects.
- It penetrates solid materials.
- It is compatible with automation.

Disadvantages

- Certain materials are damaged.
- It requires extensive safety measures.
- It requires highly trained personnel.
- The initial outlay can be costly.

The advantages of thermoradiation are the same, but the main problem of material damage, encountered in ionizing radiation, can often be avoided. In addition, a thermoradiation facility costs less because the radiation source does not have to be as large.

Although NASA selected dry heat as the best overall method for spacecraft sterilization, it became interested in ionizing radiation as a candidate process for heat-sensitive components. The continuing interest of NASA in radiation paved the way for one of the major advancements in the field of sterilization, thermoradiation.

Sandia Laboratories (Albuquerque, New Mexico) suggested that the potential value of thermoradiation justified a comprehensive evaluation. Drawing support from NASA and the Atomic Energy Commission (AEC), Sandia found that sterilization could be accomplished at much lower temperatures and radiation doses than those required by either method alone. A variety of spacecraft components that were sensitive to the high temperatures of the dry-heat process and the high doses required by ionizing radiation alone could now be sterilized. Thus, thermoradiation evolved, providing the means

to sterilize many delicate materials without damaging them.

This chapter is divided into three sections. The first section describes how the effects of radiation on microorganisms are evaluated. The remaining two sections discuss the properties of ionizing radiation and thermoradiation. Specifics about each are categorized under general physical properties, units of radiation, mode of action, factors affecting lethal efficiency, microorganism susceptibility, and effect on materials and applications.

MEASUREMENT AND EVALUATION OF RADIATION EFFECTS

For the purpose of measuring and evaluating the effects of radiation, the dose-response curve was originated (ref. 65). This curve is obtained by exposing a microbial population to radiation, and then plotting the logarithm of the fraction of surviving organisms against the radiation dose. The equation, which mathematically relates the logarithmic kill to radiation dose, is

$$\frac{N}{N_0} = e^{-D/D_0} \quad (1)$$

where N is the number of survivors, N_0 is the initial population, D is the dose, and D_0 is the mean lethal dose (the radiation dose which destroys 63% of the organisms). This relationship is illustrated in figure 22. A unit for expressing survival is the D_{10} -value, which is the negative reciprocal of the slope of the semi-logarithmic plot. The D_{10} -value is the radiation dose required to reduce the population by 90 percent. The effects of environmental conditions are evaluated by the changes they cause in the D_{10} -value. The D_{10} -value is also used to determine the total amount of radiation required for sterilization. For example, assume that a test solution contained 10,000 spores of *B. subtilis*. The D_{10} -value for this organism is 0.06 Mrad (Mrad is a radiation dose of one million rads) in water. To sterilize this population requires five D_{10} -values (0.3 Mrad) to be administered. The quantity of radiation (0.3 Mrad in this example) causing total inactivation is termed the sterilizing dose.

Experimental data are not always in agreement with the exponential model. As will be shown in the section "Thermoradiation," temperatures above 50°C during irradiation cause a greater kill than is predicted by the model. The use of the model under these

conditions requires the incorporation of a temperature-dependent rate constant.

Several theories have been developed to explain dose-response curves. In the hit theory, it is assumed that the response of the organism is caused by the absorption of radiation energy by a sensitive biological component (macromolecule) that changes its structure and function. Not all macromolecules or sites are sensitive, but when one is affected, it is termed a "hit." One hit can be lethal; however, in some cases, more than one hit is necessary to kill the cell. Hit theory aims at determining the number of hits or targets necessary to kill a microorganism. The target theory goes one step further and attempts to define the volume or size of the macromolecules. Usually, the larger the genetic molecular weight (amount of RNA and DNA) of the molecule comprising a microorganism, the more susceptible it is to radiation. Both theories account for some deviations from the semi-logarithmic curve; however, neither theory explains the effect of temperature and dose rate on the inactivation rate (D_{10} -value).

IONIZING RADIATION STERILIZATION

The effect of ionizing radiation on microorganisms was first studied in 1877. Interest in its use for sterilization was greatly stimulated after World War II, when we became more familiar with electron generators, accelerators and radioactive isotopes. However, a complete understanding of the effects of ionizing radiation on bacteria remains to be achieved.

General Physical Properties

There are two types of ionizing radiation, corpuscular and electromagnetic (ref. 66). Of the corpuscular group, only alpha particles, beta particles, and cathode rays are used for sterilization. The alpha particle is formed by the disintegration of an unstable nucleus and initially possesses high kinetic energy. As it penetrates matter, its kinetic energy is released in many consecutive ionizations. Ionization refers to the ejection or knocking out of an electron from an atom or molecule encountered by the particle as it passes through matter. The beta particle (7500 times smaller than an alpha) is a high-speed electron ejected from a disintegrating nucleus. Cathode rays are electrons that are formed by electron generators and are able to penetrate deeper in matter than the average beta

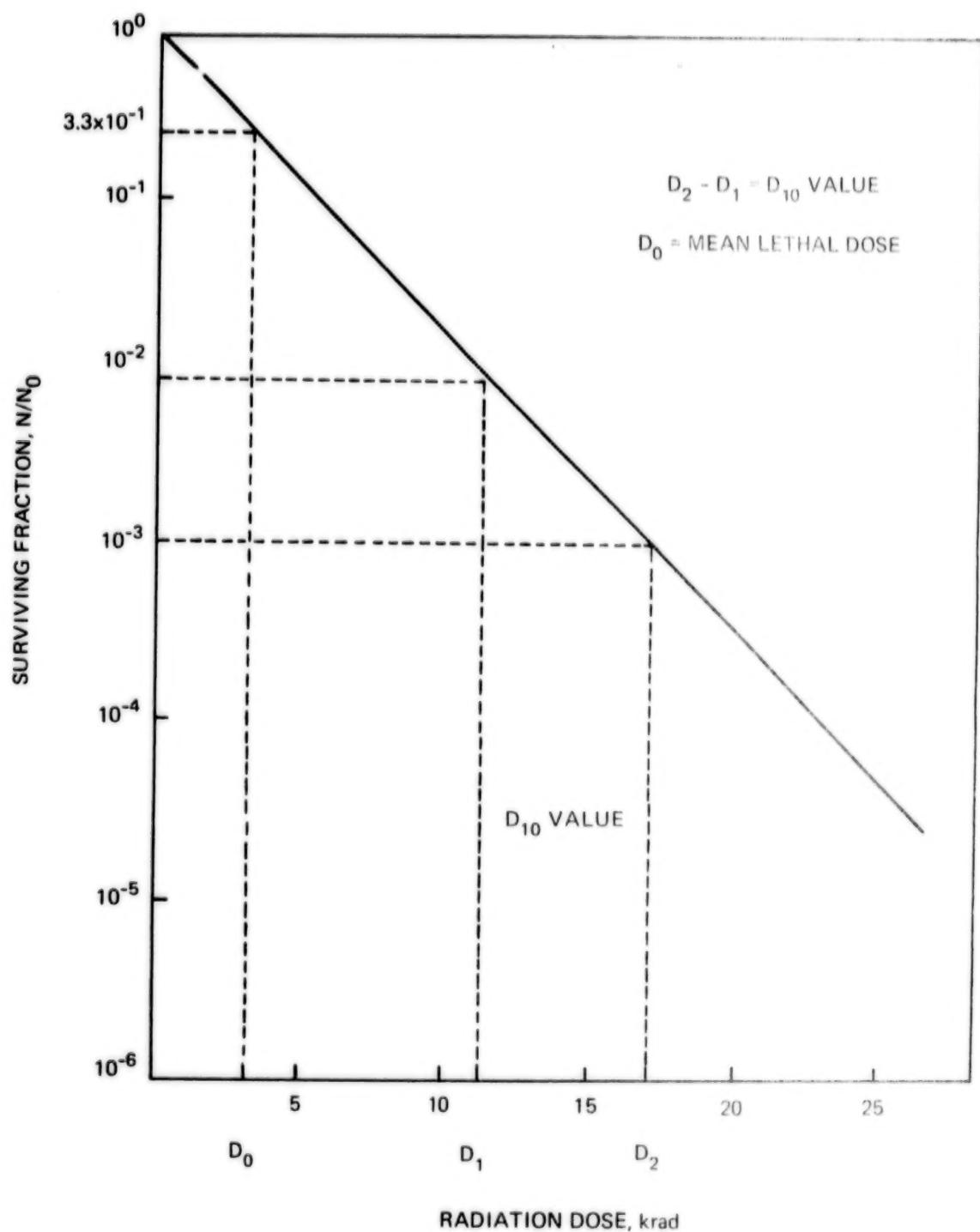


FIGURE 22. — Representative dose response curve.

particle (ref. 67). Like the alpha particle, both the beta and the cathode ray lose their kinetic energy in ionization; however, both penetrate farther than the alpha particle because of their smaller mass.

Electromagnetic radiations (ref. 68) include gamma rays, X-rays, visible light, infrared, radio waves, and ultrasonic waves (fig. 23). Gamma rays and hard X-rays are effective sterilants because they promote ionizations in matter. The other types of electromagnetic radiation are less effective because they are unable to ionize molecules. Gamma rays are identical to X-rays in nature, although they originate in a different fashion. Gamma rays originate from the transition of an atomic nucleus from an excited state to a ground state. X-rays are produced by the bombardment of a heavy metal target with high-speed electrons (cathode rays) that impact the heavy nucleus to create an unstable condition. This condition causes the emission of an X-ray. Gamma and hard X-rays react similarly with matter, and both are deeply penetrating rays.

Units of Radiation

Units have been developed that measure dose based on charge [roentgen (R)], energy absorbed [radiation absorbed dose (rad), roentgen equivalent, physical (rep)], or biological effect [roentgen equivalent, man (rem)]. The roentgen measures the ionization produced in air by the passage of X-rays or gamma radiation (ref. 69). The rep for particle radiations equals the absorption of 93×10^{-7} J (joule) (93 ergs) per gram in soft tissue. The rad corresponds to the absorption of 100×10^{-7} J (100 ergs) per gram of any medium. The rem is the absorbed dose of any ionizing radiation that produces the same biological effects in man as those resulting from the absorption of one roentgen of X-rays.

Mode of Action

The characteristic reaction of high-energy radiations is the formation of ions in the material in which they are absorbed. Free radicals and excited molecules are also created. The effect of these ions and radicals on microorganisms can be categorized as direct or indirect. In the direct case, the radiant energy is absorbed in a sensitive macromolecule, such as deoxyribonucleic acid (DNA). It then rearranges (changes its structure) to release this energy, thus

creating a primary "lesion" (ref. 65). The primary lesion could take the form of a double-strand break of the DNA helix. In this case, transcription (replication of the genetic material) would be blocked so that cell death results. On the other hand, the lesion may be of lesser importance and insignificantly affect the cell's survival ability. Lesions can also be formed indirectly, by sensitive macromolecules reacting with diffusible radicals from excited and ionized molecules in the cell's environment. The indirectly formed lesions are just as lethal and cannot functionally be distinguished from the directly formed lesions.

Factors Affecting Lethal Efficiency

Lethal efficiency refers to the extent of microbial inactivation (kill). There are a number of parameters (as detected by changes in the slope and shape of the dose-response curve) that can modify the lethal efficiency. These parameters are the gaseous environment, temperature of exposure, protective factors, physiological state, moisture content, and dose rate.

Gaseous Environment.—The presence of oxygen during exposure is a major cause of changes in the lethal efficiency. For example, spores of *Bacillus pumilus* are approximately twice as sensitive in air as in nitrogen (ref. 70). The inactivation dose for *B. subtilis* spores is proportionally reduced by the gaseous environment in the following order: oxygen > air > nitrogen > vacuum (ref. 71). With vegetative cells, the level of survival is increased two- to three-fold in the absence of oxygen. One possible explanation for these results assumes that two types of lesions are formed by the radiation. The first type is nonreparable, similar to a double-strand break of the DNA molecule. The second type is repairable (like a single-strand break in DNA) and could be corrected by the microorganism (ref. 65). The repair process is dependent on the concentration of oxygen. A higher oxygen level blocks the cell's repair mechanisms and results in a greater kill. The oxygen in the environment prevents the neutralization of radiation-formed radicals and ions, thereby increasing their chance to convert the repairable lesions into the nonreparable types. Therefore, in the selection of conditions for maximum kill, oxygen should be present (1 to 10 mg/l) in the environment (ref. 66).

Temperature.—There are two ways in which temperature changes the lethal efficiency. A higher temperature, during or subsequent to irradiation,

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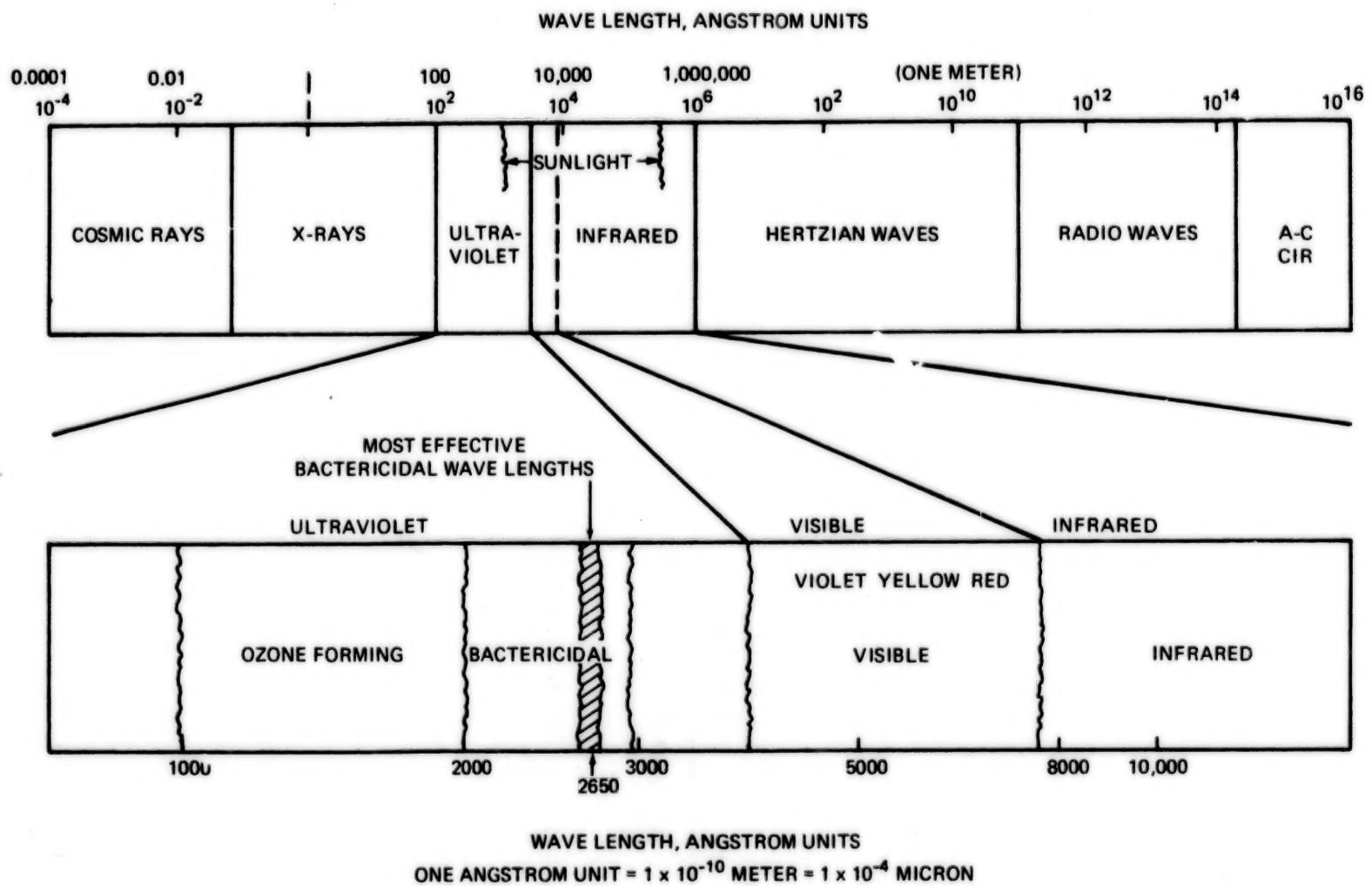


FIGURE 23.—Spectrum of radiant energy.

increases the rate of ion and radical interactions from radiation (indirect effect), and results in the formation of more lesions, both repairable and nonrepairable. This effect is discussed in the section "Thermo-radiation." The incubation temperature of the recovery medium, where lower temperatures generally increase survival, also affects the lethal efficiency. With *Escherichia coli* for example, incubation at 18°C (normally 37°C is optimal) can stimulate a hundred-fold increase in the number of survivors. Apparently, this lower temperature shifts the equilibrium normally favoring the kill response to the repair of damage. Organisms vary in the optimal temperature needed for repair, so that the selection of an incubation temperature becomes difficult when irradiating a population containing different types and species.

Protective Factors.—Organic compounds such as aliphatic alcohols, glycerol, formate, succinate, pyruvate, lactate, and propylene glycol can increase a cell's survival ability (ref. 72). Other compounds such as sodium hydrosulfite, glutathione, and sodium ascorbate can provide a similar protective effect (ref. 73). These compounds may protect by neutralizing radiation-formed radicals or by reacting with oxygen before it can enhance inactivation. Foods often contain them at concentrations which afford protection, so that a greater radiation dose must be administered when these compounds are present to achieve a level of kill equivalent to that achieved when they are absent.

Physiological State.—The physiological state of a microorganism refers to its condition relative to growth. For example, a spore is a dormant (non-growing) form of microorganism. In an environment with suitable temperature, moisture, and nutrients, the spore germinates. The germinated spore then changes into a vegetative cell capable of division and reproduction. After germination, spores are generally less resistance to ionizing radiation (ref. 72).

The growth of a population of vegetative cells is grouped into three phases: lag, logarithmic (log), and stationary (resting). In the lag phase the cells do not divide, but increase in size and replenish the internal supplies of intermediate metabolites. After this phase, they enter the log phase that consists of active division and reproduction. The resting stage is reached when the supply of nutrients and accumulation of toxic waste products inhibits the active reproduction of cells. At this stage, there is an equilibrium between the death of old cells and reproduction of new cells.

Changes in sensitivity of the population to radiation also take place in three stages (ref. 74). Resistance increases continuously during the lag phase of growth. Toward the end of the log phase, resistance falls to a minimum and climbs back to the initial resistance during the stationary phase.

The lethal efficiency or extent of kill when dose is held constant will correspondingly change with the physiological state of the cell. For most applications (with the exclusion of foods) the microbial contaminants are more frequently in the spore or resting stage, which means that a higher inactivation dose than needed in the log phase should be employed. With foods, the contaminants are likely to exhibit log-phase growth, so a lower dose may be required (ignoring the effect of protective compounds) for the same degree of inactivation achieved with spores.

Moisture.—The effect of moisture is related to the internal water content of the cell. Although some investigators agree that microorganisms irradiated in aqueous solutions (refs. 67 and 71) are less resistant than those irradiated in the dry state, others do not (ref. 72). When the indirect effects of radiation are considered, it would seem that a high internal water content would provide a greater number of radicals and ions from a constant dose, and thus would result in an increased kill. A comparison of radiation effects in dry and wet conditions shows a twofold increase in sensitivity for enzymes (invertase) and a fourfold increase in the colony-forming ability of *B. subtilis* spores in aqueous solutions (ref. 65). The greater resistance of spores can be attributed to their water content being lower than vegetative cells. Micro-organisms in the frozen state, both spores and vegetative cells, also are more resistant to radiation (refs. 65, 67, and 71). In this state, the indirect effects of radiation are reduced, since the movement and diffusion of radicals are restricted in the frozen condition.

Dose Rate.—Scientists disagree on the effects of dose rate. Theoretically, the destruction of a target is dependent on the deposition of a minimum amount of energy by the incident radiation. The same level of damage should result from a given dose, regardless of the rate at which it is administered. This probably holds true for the direct effects of radiation; however, the indirect effects can be stimulated or inhibited by oxygen, temperature, protective factors, or moisture content—conditions that frequently mask the dose-rate effect. This accounts for the conflicting

conclusions of most studies on ionizing radiation that have used high dose rates at temperatures below 50°C, but with many variations in the presence of oxygen, moisture, or protective factors. Elevated temperatures (above 50°C) stimulate the indirect effects for many systems, and the influence of dose rate becomes very pronounced. At elevated temperatures the total dose required to achieve an equivalent kill is reduced by a lower dose rate that results in part from the increased time of exposure at elevated temperature.

Microorganism Susceptibility

In general, vegetative cells usually have D_{10} -values of 50 to 100 krads or less, and spores can have D_{10} -values as much as four times higher (ref. 72). In table 6, examples of selected microorganisms and their corresponding D_{10} -values are presented (refs. 66 and 75). These D_{10} -values do not represent absolute values, since variations in conditions modify the lethal response. They are provided to give general estimates of the relative sensitivities of various groups of organisms. *Streptococcus faecium* and *Micrococcus radiodurans*, two nonspore-forming bacteria, are roughly tenfold more resistant than *E. coli*. In fact, it is not yet fully understood why, but they are usually even more resistant than bacterial spores. Spores are highly resistant probably because of their low water content. *M. radiodurans*, unlike any other organism, is resistant because of its ability to repair double-strand breaks in the DNA molecule (ref. 69).

TABLE 6.—Radiation D_{10} -Values of Selected Microorganisms

Organism	D_{10} -value, Mrads
<i>Escherichia coli</i>	0.03 to 0.06
<i>Salmonella</i> in foods	0.07
<i>Bacillus sterothermophilus</i>	0.22
<i>Streptococcus faecium</i>	0.28 to 0.56
<i>Micrococcus radiodurans</i>	0.22 to 0.88
Foot and Mouth Disease Virus	1.3
Phage	1.0
<i>Clostridium tetani</i>	0.24
<i>Clostridium botulinum</i>	0.20 to 0.30

Because of their resistance, *M. radiodurans* and *S. faecium* are commonly used as sterility indicators for radiation sterilization. A sterility indicator, as discussed in chapter 2, is a viable culture of a single species that serves to demonstrate in a positive fashion the adequacy of a sterilization process.

Materials Effects

Most studies of alterations or material changes due to radiation have been performed by the food industry. The amount of chemical change induced by irradiation is small, but it seriously hampers acceptability. Red meats change color, vitamins can be destroyed, fats can become rancid, carbohydrates can be degraded, and bleaching can occur. Certain fruits and vegetables lose their characteristic flavors. The concern for deterioration in quality and off-flavors prompted the search for methods to limit the response caused by a sterilization dose of 4 to 5 Mrads (ref. 65). Two procedures currently used are the exclusion of oxygen during irradiation, and irradiation at low temperature. Both these methods decrease the lethal efficiency, but are necessary for consumer acceptability; however, they are not applicable to all foods, since fruits and vegetables cannot withstand the freezing temperatures required for this type of sterilization without suffering tissue damage.

In aerospace applications, changes in stretch, tensile strength, brittleness, and electrical characteristics have been observed at doses (2.5 to 5.0 Mrads) proposed for spacecraft hardware sterilization. At a dose of 2.5 Mrads, polyvinylchloride can turn brown through the release of hydrochloric acid and the subsequent chemical reaction with adjacent material. Polyethylene and polystyrene are not affected at this dose. On the other hand, the physical and mechanical properties of cable insulated with rubber are deteriorated (ref. 76).

Irradiation doses of 1 to 2 Mrads (ref. 76) heighten the preservation of latex. Foam latex sponges are improved in compression and recovery performance; the resistance to tearing is decreased slightly; and the shrinkage volume of a molded sponge increases. At high doses, irradiation of wood causes varying degrees of depolymerization and decomposition. Cellulose can withstand sterilizing doses, but not repeatedly. Ordinary glass acquires a fogginess after irradiation (ref. 76).

Applications

The initial cost of an automated radiation facility is high. For example, a 1 million-curie cobalt-60

source would probably cost more than \$500,000 and the associated shielding would approach the same value. However, with this source no additional capital outlay would be required for long periods, since it has a half life of 5 years. This means that after 5 years of use, 500,000 curies would remain from the original 1 million curie source. (One curie is equal to 3.70×10^{10} disintegration/second.) Considering the long lifetime and the large number of items that can be handled, the cost of radiation sterilization is competitive with that of gaseous sterilization on a per item basis.

Current and potential applications can be categorized into three areas: pharmaceuticals, other industrials, and foods. In the pharmaceutical industry (ref. 66), large volumes of syringes, catheters, needles, catgut sutures, artificial heart valves, and other endoprostheses are sterilized every day. Other pharmaceutical products sterilized by radiation are antibiotics, hormones, steroids, vitamins, and vaccines. Penicillin and streptomycin, which are heat sensitive, have been effectively sterilized by radiation with no loss in potency. B-complex and C vitamins can be treated with a dose of 4.1 Mrads with only a slight loss in potency. Virulent influenza virus and poliomyelitis virus preparations exposed under the same conditions become nonvirulent, which is desirable for vaccines. On the other hand, certain drugs such as insulin, heparin, and morphine sulfate in solution have shown signs of degradation and loss of potency, which indicates that all drugs are not equally tolerant to the effects of radiation.

In other industries, diodes, triodes, capacitors, resistors, relays, sockets, plugs, inserts, and blocks are sterilized at a dose of 2.5 Mrads with no apparent changes in electrical characteristics (ref. 76). In every case they remain within the limits corresponding to the technical specifications of the manufacturers. For medical instrumentation that is not tolerant to normal sterilizing doses, a technique using a gas-radioisotope combination may be possible. The gas with the isotope would penetrate small cracks and fissures, diffusing into the interior of the equipment. A smaller dose of radiation (at a level appropriate to the isotope chosen) is required, since the outside source does not have to penetrate to the interior. The gas could be removed easily by evacuation. This technique could possibly be applied to any type of manufactured instrument that undergoes material or operational changes during sterilization by other procedures.

Many ongoing experiments on the sterilization of foods by radiation in the United States are receiving a great deal of attention. No practical application is permitted at present, since the U.S. Food and Drug Administration (FDA) has restricted radiation use in foods. The concern is for the potential ingestion of carcinogens (cancer-forming agents) possibly formed in foods during radiation. Therefore, current research efforts are aimed at: (1) demonstrating that foods sterilized by radiation are not a health hazard; and (2) refining methods to produce sterilized foods that are acceptable in flavor and appearance to the consumer. Long-term trials of feeding radiation-sterilized foods to animals are currently under way. Some of this research is now approaching completion, after which the FDA intends to reevaluate the restriction. At present, radiation sterilization of bacon, which maintains flavor acceptable to consumer standards, has been successfully performed at a dose level of 4.6 to 5.3 Mrads (ref. 66). Other products that have been sterilized include orange juice (ref. 77), milk and milk products (ref. 78), chicken (ref. 79), minced beef, sausage skins, and haddock.

Radiopasteurization uses the same methodology as radiation sterilization of foods, except that the dose is reduced. The goal is to destroy only a portion of the microbial flora and to increase the shelf life of the product. The fish industry is a good example of how radiopasteurization could be used advantageously (ref. 80). Fresh fish can be held for 2 weeks at 0°C to 3°C before spoilage from psychrophilic microorganisms occurs. Radiopasteurization with cobalt 60 can extend the refrigerated shelf life to 30 days. Economic benefits are accrued since low-level irradiation can be less expensive than deep freezing, and distribution costs are less. The same advantages apply to many other food products with a limited shelf life.

With fruits and vegetables, a tendency toward softening has been observed following doses in the range of 150 to 200 krads (ref. 81). For canning purposes this is not a disadvantage, but rather results in a superior final product. In addition to seafood, fruits, and vegetables, radiopasteurization has been tested on other food products. The shelf life of ground beef is extended 12 weeks with a treatment of 1 Mrad (ref. 82). In dried eggs (occasionally contaminated with *Salmonella*) a dose of 0.5 Mrad will reduce the initial population of organisms by 7 logs or "99.9999 percent" (ref. 83), and will reduce the risk of food poisoning to a much safer level.

THERMORADIATION

The successful combination of dry heat with radiation (thermoradiation) for sterilization is relatively recent. Its development can be traced to earlier studies on the effect of ionizing radiation on microorganisms, as modified by changes in temperature. The initial studies were essentially negative, because high dose rates, which masked out the complementary effects of heat, were frequently used. Until dose-response curves and the hit theory were developed, the effect of environmental conditions on the response to radiation was difficult to assess quantitatively. The first instance of the combined effect was observed during attempts to diminish the heat treatment in food sterilization. Preexposure to radiation was found to lower the requirements of the subsequent heat application (refs. 84, 85, 86, and 87). Furthermore, the effect of simultaneous application of dry heat (108°C) and gamma radiation (8 krads/hr) was greater than the additive effect of each (ref. 88).

Units of Radiation

The units of thermoradiation are basically a combination of that previously described for both components, heat (chapter 2) and radiation. Radiation dose is measured in rems, rads, and so on, and temperature is measured in degrees centigrade.

Mode of Action

The simultaneous application of dry heat and ionizing radiation kills a greater number of microorganisms than the number killed by the total of the two individual components. This greater-than-additive effectiveness is termed a "synergism." The synergistic response is based primarily on the indirect effects of radiation caused by the diffusion of radicals and ions from the environment that attack a sensitive site or target. The attack from these ions and radicals is greater with increasing temperature.

The equation describing the combined effects of heat and radiation is basically the same as the exponential equation [eq. (1)] for inactivation, with a modification for the temperature-dependent free radical attack (refs. 89 and 90). It is based on three reactions: the inactivation rate of heat alone, the inactivation of ionizing radiation alone (direct effects), and the inactivation by free radicals, which is tem-

perature and dose-rate dependent. The exponential equation for inactivation when dose rate is constant can also be expressed as:

$$N = N_0 e^{-kt} \quad (2)$$

where N is the population at time t , N_0 is the initial population, and k is the rate constant which is equal to D_{10} -value. For thermoradiation, the same basic equation holds true. Thus,

$$k = k_T + k_R + k_{TR} \quad (3)$$

where k_T is the rate constant for inactivation by temperature alone, k_R is the rate constant for radiation alone, and k_{TR} is the rate constant for inactivation by free radicals (which is dose-rate and temperature dependent). Each of these rate terms can be expressed separately as functions of temperature and/or radiation dose rate, so that this model now provides a means of describing microorganism inactivation due to heat, or ionizing radiation, or both. For example, in the application of heat alone, k equals k_T . If the inactivation is caused by radiation at ambient temperature or lower, k is approximated by k_R (target theory); however, if heat and radiation are simultaneously applied, then k is described by equation (3).

The significance of all mathematical expressions is the ability to predict a given phenomenon with a reasonable degree of accuracy. Since the sensitivity of microorganisms to radiation and increasing temperature can now be described analytically and is predictable, thermoradiation can be employed in a manufacturing process where the reliability of sterilization is a crucial consideration. Conditions such as temperature and dose rate can be selected that ensure product sterilization. They are calculated on the basis of the estimated number and types of contaminating microorganisms and the desired safety and material compatibility level.

Factors Affecting Lethal Efficiency

In thermoradiation, as in ionizing radiation alone, the lethal efficiency refers to the extent of kill. Environmental conditions such as the gas environment, temperature, protective factors, physiological state, moisture, and dose rate modify the lethal efficiency of thermoradiation sterilization. A schematic diagram of the equipment set up to test the

effects of varying environmental conditions is shown in figure 24. The notation "GIF" identifies the Gamma Irradiation Facility at Sandia Laboratories, and a view showing the remote handling equipment in the facility is provided in figure 25. Temperature is controlled to $\pm 0.5^{\circ}\text{C}$ in a recirculating air-temperature chamber in the GIF. The cobalt-60 source is placed in a corner of the cell, which is 7 feet by 8 feet by 8.5 feet high. The dose rates range from 5 krads per hour to 1 Mrad per hour, depending on the location of the sample within the cell. Moisture content is adjusted to the desired humidity by mixing dry air from a desiccant bed with moist air from a saturator and is controlled to an accuracy of ± 1 percent at room temperature. Samples exposed within the cell are aseptically removed and processed in a class 100 laminar downflow clean room (figure 26), also at Sandia Laboratories.

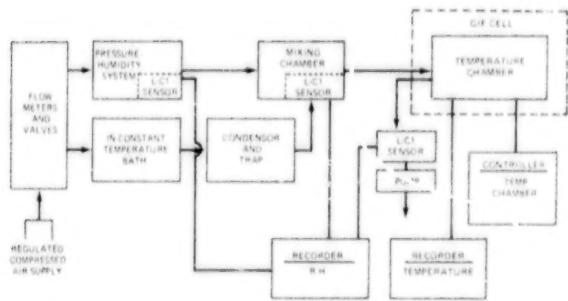


FIGURE 24. — Pressure-humidity system for controlling moisture conditions in heat, radiation or thermoradiation test space.

Gaseous Environment.—Although the presence of oxygen enhances inactivation caused by ionizing radiation, it appears that with thermoradiation, oxygen may not always cause this response. At 95°C , the D-value in nitrogen for *B. subtilis* var. *niger* spores at high dose rates (15 to 100 krads/hour) is slightly higher than in air. At the optimal dose rate of 10 krads per hour (the rate providing the maximum synergistic effect), essentially no difference in inactivation is apparent (ref. 91). In this case, thermoradiation provides a distinct advantage over ionizing radiation sterilization; because, as you will recall, the presence of oxygen during ionizing radiation, although necessary to achieve the maximum inactivation, caused unacceptable changes in food and other types of products. Because of these undesirable effects,

irradiation under nitrogen is used, even though it is less than optimal. In sterilization by thermoradiation, the maximum synergistic effects can apparently be obtained in either nitrogen or oxygen, so that oxygen-sensitive products can be effectively sterilized.

Temperature.—The effect of temperature at 95°C , 105°C , and 125°C is graphically presented in figure 27 (ref. 92). Figure 27(a) compares the effects of radiation alone (11 krads/hr), temperature (95°C) alone, and thermoradiation at 11 krads per hour. The ordinate is a logarithmic scale of the number of survivors. The abscissas are all expressed as radiation dose in krads (top scale) and time in hours (bottom scale). Figures 27(b) and 27(c) are identical except that the temperature is 105°C and 125°C , respectively. At each temperature it is clearly evident that thermoradiation killed more of the bacteria than did dry heat or radiation alone.

A comparison of the synergistic response under the specified test conditions is provided in table 7. The synergism is calculated by adding the logarithm of the number of bacteria killed by heat alone and radiation alone and subtracting this value from the logarithm of the number killed by thermal radiation. With thermoradiation, the kill ranged from 1.8 to 4.5 times greater than the summation of heat alone and radiation alone. The synergism varied for each test condition, but this difference is predicted by the model.

Representative data are summarized in figure 28. The reduction in dry heat D_{10} -value* resulting from the combined application of heat and radiation is shown. The open bar to the left of zero in figure 28 represents the D-value for dry heat alone, and to the right of zero, the value for radiation alone. The cross-hatched area represents the total combined thermoradiation treatment needed for an equivalent 1-log decrease in population. In other words, at 125°C , the D-value was reduced from 22 minutes with dry heat alone to a D_{10} of 12 minutes for simultaneous

*In thermoradiation the D_{10} -value represents the time required to inactivate 90 percent of the population at a constant dose rate and temperature, which is analogous to the dry-heat D-value (chapt. 2). With ionizing radiation alone, the D_{10} -value represents the total radiation dose required to inactivate 90 percent of the population.

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RADIATION STERILIZATION

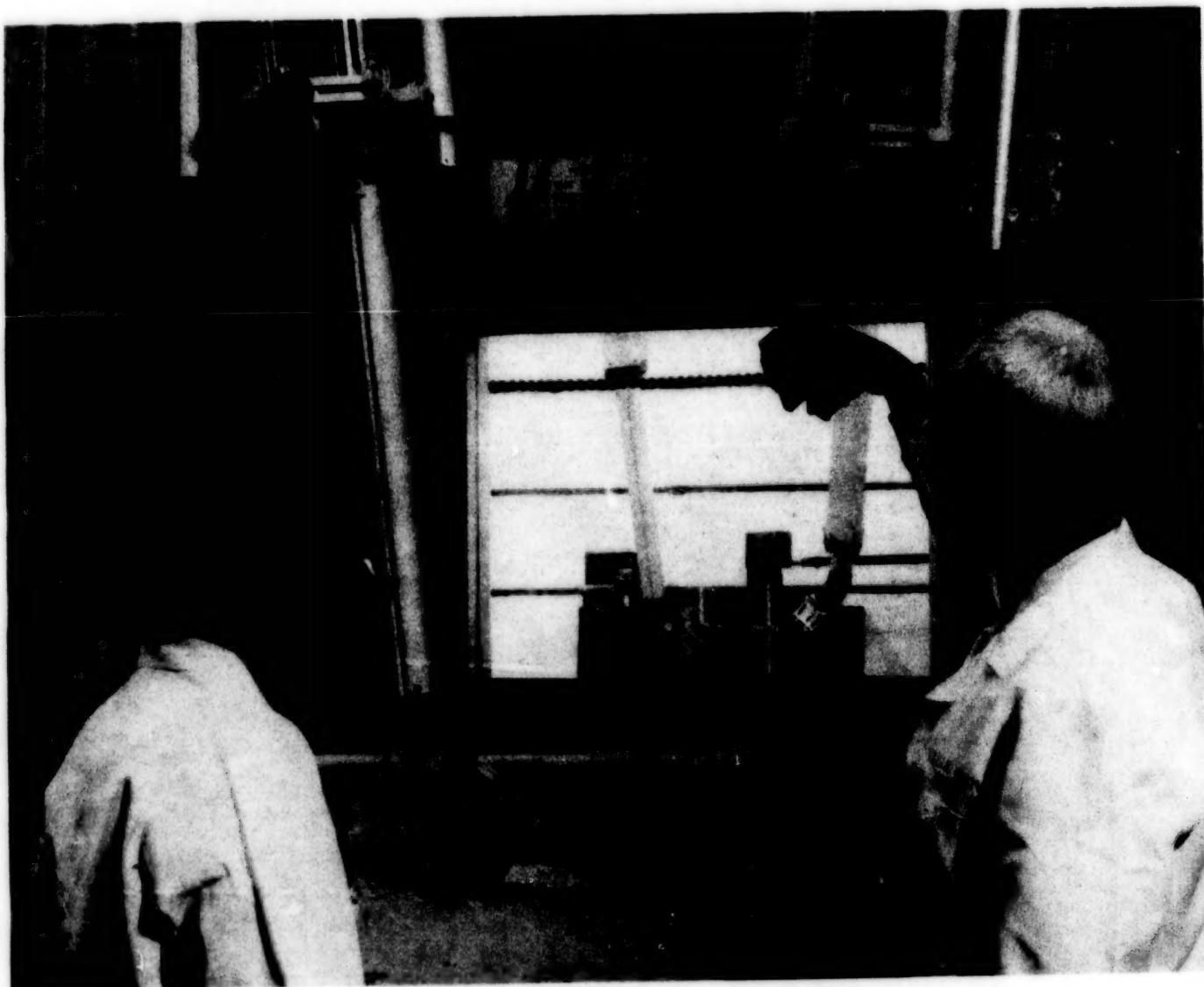


FIGURE 25. - Sandia Laboratories cobalt-60 cell.



FIGURE 26. - Sandia Laboratories laminar down flow class 100 clean room facilities.

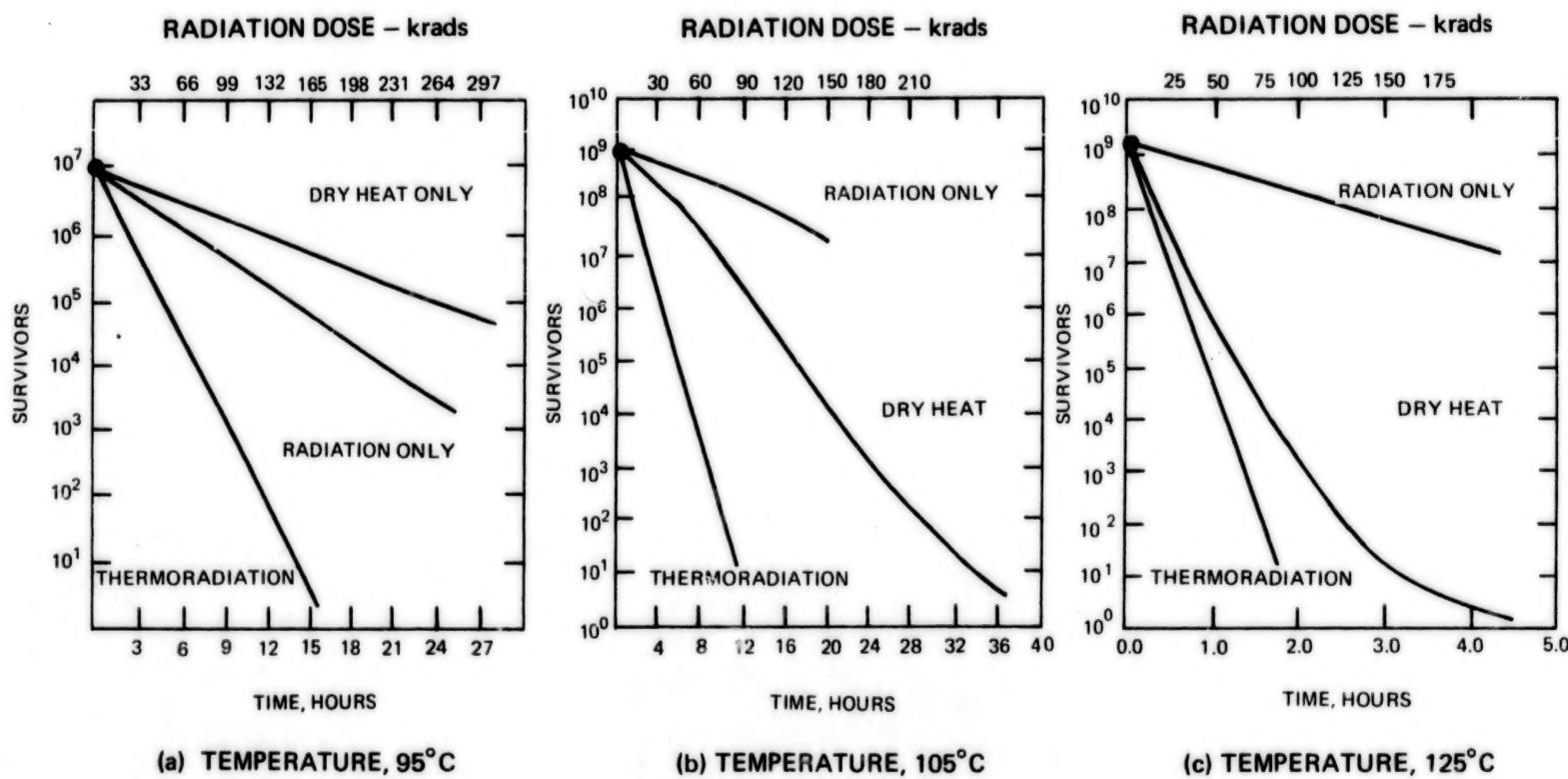


FIGURE 27. – Comparison of radiation, dry-heat and thermoradiation inactivation of *Bacillus subtilis* at 95°C, 105°C, 125°C.

TABLE 7. — Comparison of the Synergistic Response with Varying Dose Rates, Temperatures, and Time

Conditions			Logarithm of the Number of Bacteria Inactivated				
Dose (krads)	Time (hours)	Temperature (°C)	Radiation Alone (1)	Dry Heat Alone (2)	Summation of (1) & (2)	Thermo- radiation	Synergism
130	12	95	1.6	1.0	2.6	5.2	2.6
90	12	105	1.0	2.9	3.9	8.6	4.7
100	2	125	1.0	6.0	7.0	9.0	2.0

conditions—with a radiation dose of 10 krads applied during that 12-minute period. At 105°C, the D-value was reduced from 4.5 hours to a D₁₀ of 1.5 hours, with a gamma dose of 11 krads (0.011 Mrads). At 100°C the D-value was reduced from 7 hours to a D₁₀ of 2.5 hours, with a gamma dose of 19 krads (0.019 Mrads).

Protective Factors.—No data are available on the effect of aliphatic alcohols, glycols, sulfur-containing compounds, and carboxylic acids in decreasing inactivation by thermoradiation.

Physiological State.—The relative sensitivities to thermoradiation by microorganisms in different stages of growth and dormancy are also just beginning to be studied.

Moisture.—Moisture combined with heat has different effects on the lethal efficiency of ionizing radiation, as compared with the effects of heat alone. Generally, microorganisms at 20 percent to 60 percent RH, are more sensitive to heat at the lower moisture level. On the other hand, microorganism sensitivity to ionizing radiation is generally enhanced with increasing moisture. Perhaps these responses neutralize each other, accounting for the fact that different ambient relative humidities (ranging from 20 percent to 60 percent) do not appear to affect the lethal efficiency of thermoradiation for *B. subtilis* spores (ref. 91). Tests with thermoradiation at 100°C indicated that no significant changes occurred at the optimal dose rate when the humidity was varied. In systems for which this holds true, thermoradiation would be an advantageous process for sterilization, because the RH of the exposure environment would not have to be carefully controlled.

Dose rate.—The lethal efficiency is dependent on dose rate (ref. 93) at temperatures above 50°C, which is illustrated in figure 29. Constant temperature (105°C) is maintained throughout the experiment, where dose rate is plotted against the corresponding D₁₀-value. Small increases in the dose rate, in the range of 1 to 12 krads per hour, cause large reductions in the D₁₀-value. Beyond this range there is only a small reduction in D₁₀-value as the dose rate is further increased. The dose-rate dependency is actually a nonlinear relationship, where the k_{TR} rate constant is based on a power function. In other words, the effectiveness of radicals is increased with increasing dose rate up to a certain level, where perhaps radical generation equals radical depletion during the inactivation process. At this level, more radiation does not substantially increase their effectiveness. Decreasing the dose rate lengthens time at a given temperature, which in itself increases inactivation, but does not entirely explain the enhanced effects.

The significance of these data is typified by the difference in total doses required for a given population reduction. Considering a 99.99 percent inactivation at 105°C, a high dose rate (36 krads/hour) requires a total dose of 90 krads to be administered. A low dose rate (2.6 krads/hour), however, requires only 21 krads to achieve the same result. This result indicates another measure of flexibility that can be used advantageously. Usually, material tolerance to ionizing radiation is based on total dose. Since total dose is adjustable, a choice can be made between a low dose rate for radiation-sensitive materials, or a high dose rate and short exposure time for radiation-resistant materials.

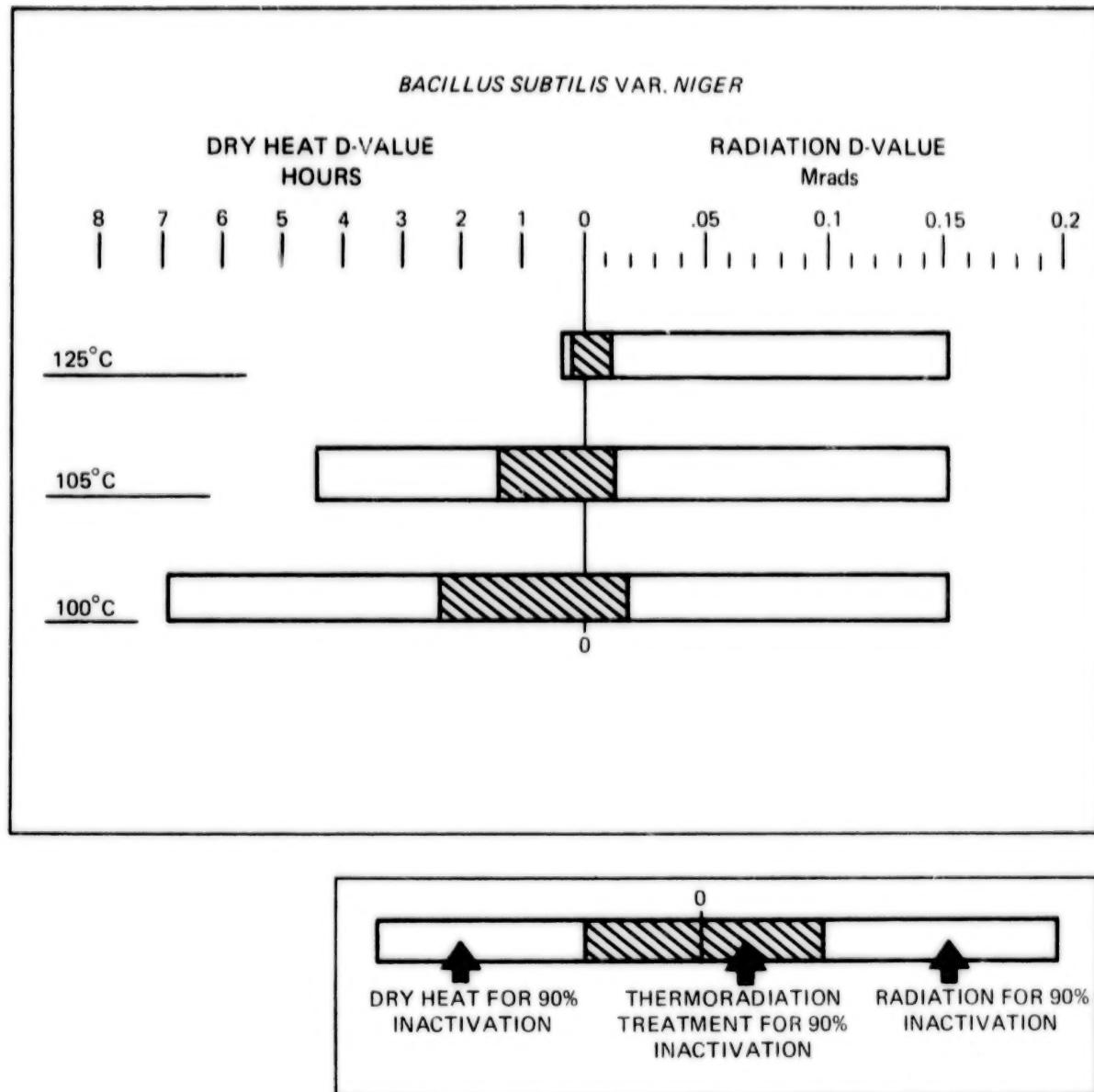


FIGURE 28. – Comparison of D-value for radiation alone, temperature alone and thermoradiation of *Bacillus subtilis* var. *niger*.

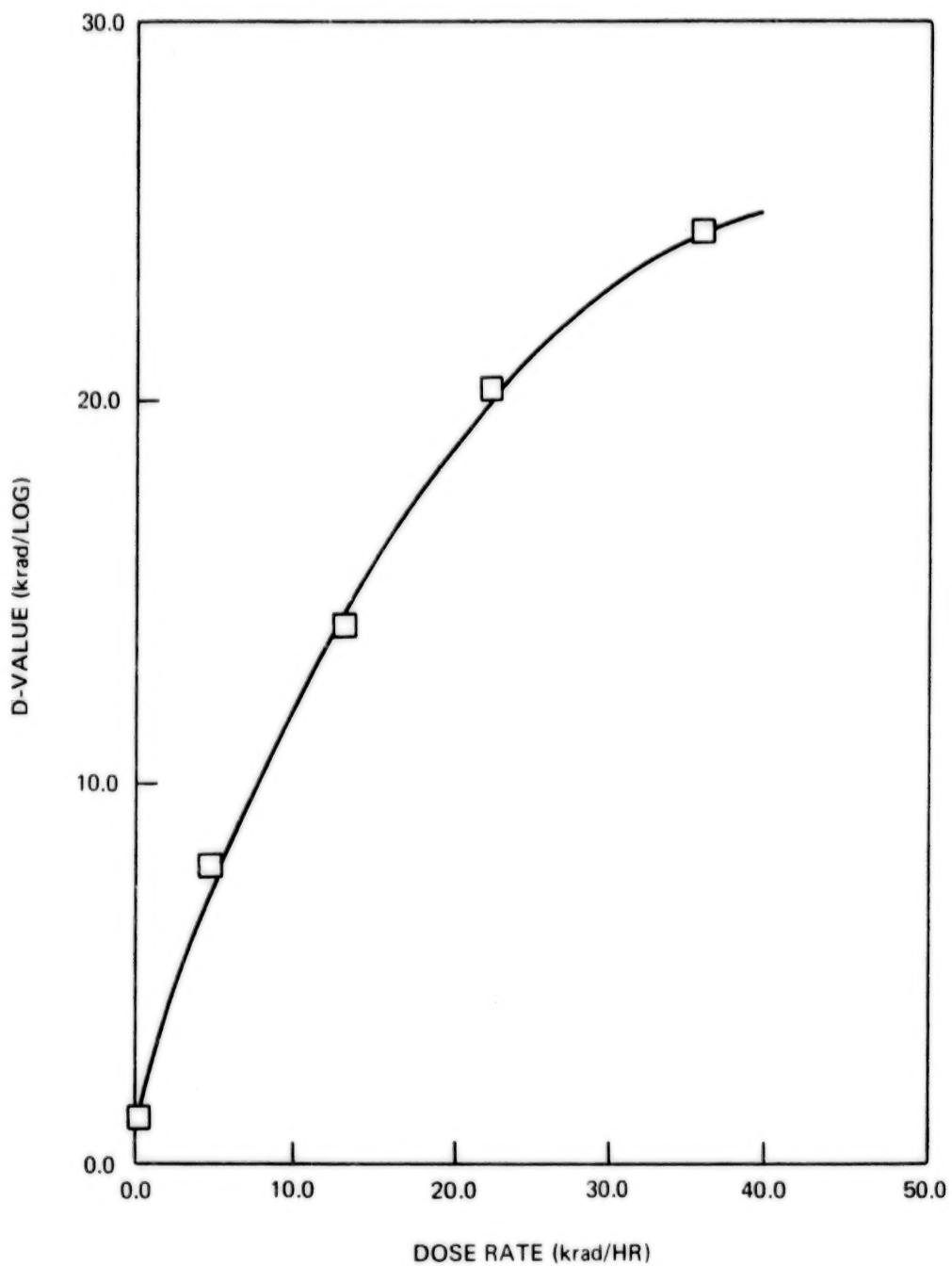


FIGURE 29. — Effect of radiation dose rate on the corresponding D-value at 105°C *Bacillus subtilis*.

Microorganism Susceptibility

To date, microorganisms tested in the thermoradiation environment include bacteria, viruses, phage, and *ascaris lumbricoides* parasite ova. The bacteria tested were *B. subtilis* var. *niger* spores, *Clostridium botulinum* spores, *Bacillus megaterium*, *Bacillus cereus*, and *B. pumilus*, coliforms, fecal strep, and salmonella species. Newcastle Disease virus, Tobacco Mosaic virus, Ø X174, T1 phage, T2 phage, and T4 phage, and attenuated polio viruses represent the animal, plant, and bacterial viruses exposed to thermoradiation. Reduction of the required inactivation dose of ionizing radiation alone was achieved by thermoradiation. It is interesting to note that viruses that are highly resistant to ionizing radiation were readily inactivated by thermoradiation. Studies indicate that the same degree of inactivation can apparently be achieved by thermoradiation at doses and temperatures lower than those required either by ionizing radiation or heat alone. Consequently, its use is more advantageous for a wider range of organisms.

Materials Effects

The tolerance of materials to thermoradiation cannot be stated out of hand. In the first place, there are numerous possible combinations of temperatures and radiation doses. Secondly, the total radiation dose applied is variable, depending on the dose rate; therefore, the categorical description of each of these possible combinations on a variety of materials becomes an enormous task. The materials effects must be studied for the particular combination of temperature, time, and dose rate chosen.

The primary reason for the development of thermoradiation was to reduce the stress applied to materials during harsh sterilization. The method provides the flexibility of sterilizing a given item by the combination of heat and radiation that results in the least stress or the fewest undesirable side reactions. The sterilization cycle can be custom-designed to fit the characteristics of the material being treated. For example, many options are now available to reduce a population of *B. subtilis* var. *niger* spores on an exposed surface by a factor of 10^{12} . One option is dry heat (105°C) with a total sterilization time of 54 hours. A second option is thermoradiation (105°C) at 12 krads per hour (D_{10} -value of 1.1 hours) for a

total time of 13 hours, and a total dose of 144 krads. The third option for less radiation-sensitive material is a dose rate of 36 krads per hour. At this rate, the D_{10} -value is 0.7 hours, resulting in 8.4 hours for sterilization at 105°C , with a total dose of 300 krads. This flexibility permits the patterning of sterilization cycles around the material being sterilized to ensure the highest product quality obtainable.

Thermoradiation can also have beneficial effects on materials, such as the annealing of harsh radiation effects by heat in MOS solid state devices.

Applications

Because thermoradiation is a fairly new technology, applications are currently being researched, and many are near commercial development. For example, research in sewage sludge pathogen reduction, to permit land application to food crops and ruminant animal refeeding, has advanced to the point where a 20,000 gallon-per-day system will soon be installed in a municipal sewage plant. A plant to treat the dried compost (50 percent solids) equivalent to 50,000 gallons/day of sludge is also being planned. Other applications under active research and development include chemical detoxification of liquid pollutants, and sterilization of pharmaceuticals, foods, and water (in sensitive processes such as in fish hatcheries).

Spacecraft components such as batteries, high-value Mylar capacitors, vidicon tubes, tantalum capacitors, solid propellants, photometers, and other scientific instruments, are degraded when exposed to high temperatures or high radiation doses. With thermoradiation, sterilization of these types of components now appears to be possible without operational performance losses. In addition, any type of equipment or instrumentation, such as microscopes, spectrophotometers, pumps, potentiometers, pH meters, amplifiers, analyzers, cardiac-flow monitors, blood oxygenators, cameras, kidney machines, and physiological monitoring equipment probably can be sterilized by thermoradiation with a high probability that operational loss will not occur.

Certainly, the pharmaceutical industry can use thermoradiation for sterilization of antibiotics, vaccines, hormones, steroids, and vitamins.

One medical application being studied is the inactivation of hepatitis virus in blood. To date,

results have indicated that lower temperatures (at a given dose rate) are required for the synergistic inactivation of biological systems with a higher nucleic acid (DNA) content. Blood components have a relatively low amount of nucleic acid in comparison to viruses. The causative agent of hepatitis, a blood disease, is probably viral in character; therefore, by selecting the proper conditions of temperature and dose rate, one may be able to inactivate the hepatitis causative agent by thermoradiation without damaging the blood or blood fractions.

This same property of thermoradiation—namely synergism at lower temperatures for systems with a higher nucleic acid content—suggests another medical application. Human cells contain much more DNA than viruses or bacteria, indicating that a synergistic kill of human cells could possibly occur at a much lower temperature. Although data at this time are almost nonexistent, those available suggest that the temperatures associated with synergistic inactivation of human cells by thermoradiation lie within a few degrees centigrade of body temperature. If it were possible to collimate heat, a tumor could be heated from one direction and irradiated from another. The intersection of heat and radiation could destroy the cancerous cells because of the synergistic effect; whereas healthy tissue would be exposed to only one component and would not be seriously affected. With this rationale, dose rates and temperatures would be selected with individual effects on healthy tissue that are small compared with the combined effect in the tumor area. Computer programs are presently being developed to optimize the focusing of heat to body tumors which are not easily heated by other means.

The food industry may benefit the most from the new thermoradiation technology. This statement is based on the assumption that ionizing radiation sterilization of foods does not represent a health hazard and that it can be used without jeopardizing the safety of the consumer. Processors could select the optimal combination of heat and radiation for maintaining product texture, flavor, appearance, and nutritional value. Radiopasteurization with a low-temperature heat treatment could possibly extend the shelf life of fresh fish, meats, vegetables, and fruits beyond that which is now possible, with minimal changes in the desirable "fresh" characteristics of the food.

Another important potential use for radiation stems from the growing concern about the toxicity of

chemical fumigants used for disinfestation of fruits and vegetables in interstate and international commerce. Continued usage of these chemical fumigants to meet quarantine requirements is in no way assured. Studies currently under way are using low level doses (75 krads) to disinfest papaya of fruit fly ova and larvae. Feeding studies of irradiated papaya have been completed and a petition for process approval is being prepared. Citrus fruits, pomafruits, and vegetables will be investigated to judge the applicability of low-level radiation doses for the disinfestation of agricultural goods.

An important milestone for food irradiation processes was passed in September of 1976 when the International Atomic Energy Agency (IAEA) announced that a group of experts, convened to study the results of years of research by many nations, found radiation-treated potatoes, wheat, chicken, papaya, and strawberries to be "unconditionally safe" for human consumption. Three foods, rice, fish, and onions, were given provisional approval.

Another possible application is in wastewater treatment. In the conventional primary-secondary treatment of wastewater, one objective is to remove bacteria and viruses. With the current methods, however, the overall degree of removal is in question (ref. 94). Specifically, results indicate that chlorination of secondary effluent, as it is now practiced, does not produce virus-free effluent (ref. 95). Treating such effluent with thermoradiation could be much more effective in the inactivation of both bacteria and viruses.

The reliable removal or inactivation of bacteria and viruses from treated wastewater is growing more important for two reasons: (1) the waters that receive treatment-plant effluent are being used increasingly for recreation, particularly in large urban areas; and (2) direct reuse of treated water by humans, as proposed for the future, will require means of preventing the number of organisms from building up in recycled systems (ref. 94).

Irradiation of sewage sludge is attracting worldwide attention (ref. 96) for several reasons. First, the removal and disposal of sludges from primary and secondary treatment plants represent nearly half of plant operation costs, even though the sludge comprises only 1 to 2 percent of the total waste volume in the plant. Reduction in the radiation source requirement would substantially reduce costs. Second, pathogen reduction, so that sludge may be safely

used, is a primary concern. It has already been shown that pathogen reduction in sewage sludges, by use of thermoradiation, has been enhanced by bubbling oxygen through the sludge just prior to and during thermoradiation (ref. 97).

Additional benefits anticipated as a result of radiation treatment of sewage sludge are: chemical toxicant reduction, increased settling rates, odor reduction, and increased mineralization when used for fertilization.

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CHAPTER 4

Chemical Disinfection

"And washed it in permanganate with carbolated soap."

INTRODUCTION

There are many types of chemical disinfectants or bactericides that are widely used in hospitals, hotels, and motels; and in the paint, food, and pharmaceutical industries. They are used every day around the house as laundry bleach, household cleaners, and as antiseptics for cuts. Disinfectants are also used in swimming pools, sewage plants, waste water effluents, and to make water potable.

However, chemical disinfection as discussed in this chapter, deals primarily with the chemical cleaning or disinfection of spacecraft parts. An attempt is made to review some of the more recent work done on the disinfection of interplanetary spacecraft, and to describe it so that the techniques researched can be applied to the nonspace industries. Some of the most exciting and significant recent advances in this field have resulted from investigations both in the United States and the U.S.S.R. These advancements are: the improvement in the biological activity of surface-decontaminating agents; the extension of the use of silver's oligodynamic action; the development of self-sterilizing coatings and encapsulants; and the discovery of the synergistic effects of physical and chemical agents.

NASA first became interested in bactericidal solutions as a means of preventing the accumulation of microbial burden on spacecraft to reduce the severity of the final sterilization. Surfaces (floors, walls, bench tops, etc.) of assembly and test clean rooms are regularly decontaminated to reduce the accidental transfer of particulate contamination and microorganisms onto the spacecraft or its components. Ethanol and isopropanol, two of the solutions used to clean oil films and residues from spacecraft parts after manufacture, are to some extent bactericidal and have been applied on a number of spacecraft.

Strictly speaking, the term "bactericide" refers to a substance that kills bacteria; however, in this chapter it is used synonymously with microbiocide, a substance that destroys bacteria, spores, fungi, and viruses. Antibiotics and preservatives are omitted from this discussion since these areas have not been pursued in great depth by NASA-supported research.

Disinfectants are the oldest group of antimicrobial agents, and are cheap enough to be used on a mass scale. Since they are to attack microbes on inert surfaces and in the presence of only a slight amount of organic material, disinfectants must act on all microorganisms in highly concentrated solutions and preferably within a short time (ref. 98).

A disinfectant is a chemical agent that frees from infection; i.e., destroys disease germs or other harmful microorganisms (but not, ordinarily, bacterial spores) and is commonly applied to inanimate objects. The official definition of the word "disinfection" adopted by the American Public Health Association is as follows: "Disinfection—killing of pathogenic agents by chemical or physical means directly applied" (ref. 99).

There is an important difference between disinfection and sterilization. A disinfectant may be defined as an agent which destroys disease-causing microorganisms. When the disinfecting agent becomes powerful enough to kill all forms of microbial life, including highly resistant bacterial spores, it is then called a sterilizing agent, sporicide, or sterilant. Although many chemicals used alone or in combination are officially labeled as germicidal agents or disinfectants, very few are recognized as sterilants.

A large number of disinfectants or germicides are available under a variety of trade names; however, most may be classified as halogens, acids or alkalies, heavy metal salts, quaternary ammonium compounds, phenolic compounds, and aldehydic compounds.

No single chemical antimicrobial agent is best or ideal for any and all purposes. This is not surprising in view of the variety of conditions under which agents may be used and the many types of microbial cells to be destroyed. An ideal antimicrobial disinfectant agent would have to possess a formidable array of specific characteristics.

FACTORS AFFECTING THE EFFICIENCY OF DISINFECTANTS

There are many factors that affect the antimicrobial efficiency of disinfectants. They include the environmental temperature, concentration of the bactericide, presence or absence of surface organic matter, compatibility of the bactericide with the surface material, and the susceptibility and length of exposure time of the surface microorganisms to the bactericide (ref. 100). A brief discussion of each of these factors follows:

- Temperature can modify the extent of kill. A low temperature will slow or stop the action of the bactericide. High temperatures can also stop the killing action by evaporating or degrading the bactericide. What is a high temperature for one chemical compound may be low for another, so that the relative terms of high and low are difficult to generalize. Nevertheless, in the temperature range where the bactericide is effective, a higher temperature will usually increase the biological activity and reduce the necessary exposure time.
- Concentration also modifies biological activity. As a general rule, the stronger the bactericidal solution, the greater is the killing action. Alcohol is one notable exception, with 70 percent ethyl alcohol generally being more effective than 95 percent.
- Presence of organic matter, such as soil, blood, or other extraneous materials, can reduce the effectiveness of a chemical bactericide. This reduced effectiveness occurs either because the material prevents the germicide from contacting the microorganisms, or because the material chemically inactivates the bactericide before it has an opportunity to act upon the microorganisms. For decontamination to be most effective, the surface of the material should be cleaned before disinfection.

- Material compatibility refers to the chemical reaction of the bactericide with the material being decontaminated. Different types of materials can interfere with the action of the bactericides by creating oxidizing, reducing, alkaline, or acid conditions. In other situations, it is necessary to protect the material from the chemical action of the bactericide.
- Microorganism susceptibility to destruction depends on the characteristics of the bactericide. Some are highly effective only against vegetative cells, whereas others are effective against spores; however, very few bactericides are effective against spores. In addition, very few bactericides are versatile enough to be effective against all types of organisms.
- Time of exposure is dependent on the number of surface contaminants and the preceding factors. The greater the number of organisms to be killed, the greater the exposure time necessary to obtain the desired kill.
- The pH of the solution is also important since various antimicrobial agents require different optimum pH ranges for effective results. The time needed for each agent to obtain antimicrobial activity in a given solution may vary from seconds to hours.

All of these factors have a bearing on the rate and efficiency of antimicrobial destruction.

MAJOR GROUPS OF CHEMICAL ANTIMICROBIAL AGENTS

The most commonly used antimicrobial agents employed in various NASA-supported research projects are:

- Phenol and phenolic compounds
- Alcohols
- Halogens
- Heavy metals and their compounds
- Dyes
- Soaps and detergents
- Quaternary ammonium compounds
- Acids and alkalies
- Hydrogen peroxide (oxidants)
- Aldehydes
- Gaseous chemosterilizers (ETO, etc.)

Phenol And Phenolic Compounds

Phenolic substances may be either bactericidal or bacteriostatic, depending upon the concentration used. Some phenolics are highly fungicidal, but they are not usually sporicidal. The antimicrobial activity of phenolics is reduced at an alkaline pH and in the presence of organic material. The germicidal property of phenol is significantly reduced by low temperatures and soaps.

These compounds probably act by denaturing cell proteins and damaging cell membranes. Some, particularly hexylresorcinol (S.T.37), greatly reduce surface tension; and this property undoubtedly contributes to their antimicrobial action (ref. 101).

Cresols are several times more germicidal than phenol; the three different forms, o-, m-, and p-cresol, are approximately equal in bactericidal activity. In general, reference to cresol usually means a mixture of the three forms such as Tricresol. The cresols have limited solubility in water but readily form emulsions in liquid soaps and alkalies; however, they are now being replaced by another phenolic compound, o-phynylphenol. Like phenol, these compounds are used for the disinfection of inanimate objects.

Alcohols

As a chemical group the alcohols possess many desirable features for disinfection. They have a bactericidal rather than bacteriostatic action against vegetative forms, they are relatively inexpensive, and are usually easily obtainable. They also have a cleansing action and readily evaporate. A concentration of 50 to 70 percent alcohol is recommended to be most effective for disinfection.

Alcohols in general are bactericidal in one of three modes of action: denaturing proteins, interference with metabolism, and lytic actions. In addition to being protein denaturants, alcohols are also lipid solvents. Their germicidal activity is in part the result of cell membrane damage (ref. 102). There is a progressive increase in germicidal power as the molecular weight of alcohols increases, with the higher alcohols—propyl, butyl, amyl, and others—being more germicidal than ethyl alcohol.

Ethyl alcohol is extensively used as a skin disinfectant; but it cannot be relied on to produce a sterile condition, for concentrations which are

effective against vegetative cells are impotent against bacterial spores. Methyl alcohol is less bactericidal than ethyl alcohol.

Halogens

Disinfectants of this group of nonmetallic elements contain iodine, chlorine, bromine, or fluorine. Of these, iodine and chlorine are the most widely used.

Iodine is only slightly soluble in water, but is readily soluble in alcohol and aqueous solutions of potassium or sodium iodide. Iodine is traditionally used as a germicidal agent in a tincture of iodine. Common tinctures of iodine are: 2 percent iodine plus 2 percent sodium iodide diluted in alcohol, 7 percent iodine plus 5 percent potassium iodide in 83 percent alcohol, and 5 percent iodine and 10 percent potassium in aqueous solution. A relatively new class of iodine compounds are called iodophors. In these organic compounds, the iodine is loosely combined with some surface-active agent, with the iodine being released slowly.

Chlorine, either in the form of gas or in certain chemical compounds, represents one of the most widely used disinfectants. Hypochlorites and chloramines are the most common chlorine compounds. Calcium and sodium hypochlorite are popular compounds widely used domestically and industrially. They are available as powders or liquid solutions and in varying concentrations, depending upon the intended use. Chloramines are characterized by the replacement of one or more of the hydrogen atoms in an amino group of a compound with chlorine. One advantage of the chloramines is that because they are more stable than hypochlorite, they prolong the release of chlorine and its disinfecting action (ref. 103).

It has been suggested that the mechanism by which iodine and chlorine exert their antimicrobial activity is to involve the direct halogenation of (attachment of chlorine or iodine to) tyrosine units of enzymes and other cellular proteins, thus altering their structure and function. Iodine is also an oxidizing agent, which also may account for its antimicrobial action.

The antimicrobial action of chlorine appears to be via the action of hypochlorous acid that forms when free chlorine is added to water. Chlorine also acts in the direct halogenations of cellular proteins.

Heavy Metals And Their Compounds

Most of the heavy metals, either alone or in certain chemical compounds, exert an antimicrobial effect upon microorganisms by combining with cellular proteins to denature them. The most effective are mercury, silver, and copper. Other heavy metals exert only slight germicidal action on bacteria.

The most prominent heavy metal compounds are those of mercury and silver. Some of the more commonly used compounds of mercury are Merthiolate (1:1000 isotonic solution), Metaphen (1:2500 aqueous solution), Merphenyl nitrate (1:1500), Mercurochrome (1:50) aqueous solution, and Mercuric chloride (1:1000 aqueous). The most common forms of silver are silver nitrate, silver lactate, silver picrate, and other colloidal preparations of silver or insoluble silver compounds.

The lethal effect exerted upon bacteria by some heavy metals in extremely small concentration; i.e., at the microgram level, is designated "oligodynamic action." This phenomenon can occur in concentrations of a few parts per million of the metal. The oligodynamically active metals, particularly silver, have been used in a variety of applications to control microbial populations, such as in the treatment of water supplies and the impregnation of fabrics and ointments, making them bacteriostatic or bactericidal.

Dyes

There are a number of dyes that exhibit antimicrobial action. Dyes are used as disinfectants only to a limited degree and are generally more efficient against gram-positive than gram-negative organisms. The acridine dyes (e.g., acriflavine) have a wide spectrum of antibacterial activity which is most effective against gram-positive types (ref. 104).

The specific manner by which dyes inhibit microorganisms is not completely understood. It is assumed that their action is through combination with cellular macromolecules; for example, acridine dyes are known to bind to nucleic acids to inactivate them.

Soaps and Detergents

This class of disinfectants acts primarily by being surface-tension depressants or wetting agents.

The real value of soaps lies in the mechanical removal of microorganisms. Soaps reduce surface

tension and thereby increase the wetting power of the water in which they are dissolved, thus lysing the organisms. Soapy water has the ability to emulsify and disperse oils and dirt. The microorganisms become enmeshed in the soap lather and are removed by the rinse water. Various chemicals can be incorporated into soaps to enhance their germicidal activity. With the incorporation of a germicidal agent, the killing action occurs because of depressed surface tension and the specific action of the germicide.

There are two basic types of detergents, anionic and cationic. The anionic form ionizes with the detergent property resident in the anion; for example, sodium lauryl sulfate. The cationic form ionizes with the detergent property resident in the cation; for example, catylpyridinium chloride. There is also a third type of detergent that is nonionic, but it is not significantly antimicrobial.

Quaternary Ammonium Compounds

The cationic detergent compounds are regarded as more germicidal than the anionic compounds. The most important cationic compounds are the quaternary ammonium salts. The bactericidal power of the quaternaries is exceptionally effective against gram-positive bacteria and quite active against most gram-negative bacteria. Quaternaries also have the ability to manifest bacteriostatic action far beyond their bactericidal concentration. These concentrations range from dilutions of one part in a few thousand to one part in several hundred thousand. Quaternaries have been shown to be fungicidal as well as destructive to certain pathogenic protozoa; but viruses appear to be more resistant than bacteria and fungi.

The mode of action of the quaternary compounds is not precisely understood. It is thought that they inactivate certain enzymes of the microorganisms through their capacity to combine with and denature proteins; however, they probably act by disruption of cell membranes.

Acids and Alkalies

The acids and alkalies limit microbial growth by lowering or raising the pH of the growth environment. A change in the pH required for growth results in cessation of metabolism and death.

The acid compounds are in the form of either mineral acids (e.g., hydrochloric and sulfuric acids)

or organic acids (e.g., perchloric acid). The mineral acids function according to the degree of dissociation of ions and hence are dependent on the final hydrogen-ion concentration. The organic acids are somewhat different in that not only is their antimicrobial activity dependent on hydrogen-ion concentration, but also on the nature of the molecule.

The disinfectant action of alkalies is also dependent upon dissociation and the resulting concentration of hydroxyl ions. However, the antimicrobial activity of the alkalies is also due to the toxic effect of the metallic ions (which can be bacteriostatic or bactericidal themselves) usually associated with them.

Strong acids and alkalies are sporicidal, but their application is limited because of the corrosive nature of such concentrated solutions. In general, acids are more effective disinfectants than alkalies.

Oxidants

The oxidants owe their antimicrobial activity to their ability to oxidize cell components. Of these, hydrogen peroxide is the most widely used. It is viricidal as well as bactericidal. Other oxidizing agents used for chemical disinfection are zinc peroxide and potassium permanganate.

Aldehydes

The most important compound in this group is formaldehyde. Although formaldehyde is normally used in gaseous form, it can also be used effectively in an aqueous solution called formalin. Formalin usually contains 37 to 40 percent formaldehyde.

Formalin is used to kill not only vegetative cells but also spores. It is very effective as a bactericidal agent and is also an efficient sporicidal and fungicidal agent. Formalin was used to sterilize the Radioisotope Thermoelectric Generator (RTG) cooling loop before the launch of the Vikings.

Another chemical disinfectant is alkalinized glutaraldehyde. Its sterilizing properties were discovered in the early sixties by Pepper and Lieberman (ref. 105). The commercial formula developed by these authors consisted of a 2 percent aqueous glutaraldehyde solution buffered by suitable alkalinizing agents (generally 0.3 percent sodium bicarbonate) to reach a pH of 7.5 to 8.5.

Acid glutaraldehyde in aqueous solutions is a faster sterilant than alkalinized glutaraldehyde. The sterilant activity, however, is strictly dependent upon the

temperature of the solution. A sharp increase in sterilant activity was especially noticed over the entire 45°C to 70°C temperature range.

Gaseous Chemosterilizers

This type of disinfection or sterilization will be covered in chapter 5, "Gaseous Sterilization." Gaseous chemosterilizers that are in use today are ethylene oxide, formaldehyde, B-propiolactone, and methyl bromide.

Table 8 is a comparison of the major classes of commonly used disinfectants. It shows some of the advantages and disadvantages of each and their relative efficiencies against bacteria, fungi, and spores (ref. 106).

SURFACE DISINFECTION

The search for superior bactericidal solutions for decontamination of surfaces has been a continuing effort in the U.S. NASA program and apparently also in the Soviet program. The ideal bactericide kills bacteria and bacterial spores, fungi and fungal spores, and viruses. It is nontoxic to humans, acts rapidly, does not damage materials and components, does not leave residues, is not inactivated by the treated material, and is simple to administer. None of the compounds in table 8 completely satisfy this description.

The importance of these factors varies depending on the nature of the application. In the decontamination and cleaning of spacecraft hardware surfaces, the lack of material damage, strong bactericidal activity, lack of residues, and rapid action are very important. In the United States, isopropanol and ethanol have been the compounds of choice because they do not leave residues or damage most materials, even though they are not effective sporicides or fungicides.

Hydrogen peroxide (H_2O_2), although long known for its bactericidal properties, is now being intensively evaluated by Russian investigators. Renewed interest in H_2O_2 for surface decontamination was stimulated by the preliminary results obtained from the combination of this compound with an anionic surfactant (ref. 107). It was found that the hydrogen peroxide-surfactant solutions were effective against vegetative microorganisms (at a 3 to 5 percent concentration), spores (6 to 15 percent), and viruses (1 to 5 percent). At these concentrations, the solution was reported to not cause significant material damage and not leave

TABLE 8. - *Comparison of Selected Bactericides*

Disinfectant	Advantages	Disadvantages	Bactericidal Efficiency	Fungicidal Efficiency	Sporicidal Efficiency
Phenolic Compounds	<ul style="list-style-type: none"> • Nonspecific concerning bactericidal and fungicidal action • Not inactivated by organic matter • When boiling water would cause rusting; the presence of phenolic substances produces an antirusting effect • Solutions may be heated 	<ul style="list-style-type: none"> • 2% solutions of phenol compounds (N.F.) produce pungent odor at elevated temperature 	<ul style="list-style-type: none"> • Excellent 	<ul style="list-style-type: none"> • Excellent 	<ul style="list-style-type: none"> • Not effective against resistant bacterial spores at room temperature
Alcohols	<ul style="list-style-type: none"> • Inexpensive • Easily obtainable • Safe • Possess cleaning action • Acts quickly • Evaporates readily • Leaves no residue 	<ul style="list-style-type: none"> • Flammable • Strong concentration required 	<ul style="list-style-type: none"> • Effective against vegetative forms 	<ul style="list-style-type: none"> • Effect on fungal spores not reliable 	<ul style="list-style-type: none"> • Little effect on bacterial spores by themselves
Iodine and Iodophores	<ul style="list-style-type: none"> • Highly reactive • Low tissue toxicity • Kills immediately rather than by prolonged period of stasis 	<ul style="list-style-type: none"> • Corrosive • Some organic and inorganic substances neutralize effect • Certain iodine compounds stain materials 	<ul style="list-style-type: none"> • Sufficiently high to permit use as an emergency sterilizing agent for surgical instruments 	<ul style="list-style-type: none"> • Recognized as having high fungicidal and fungistatic efficiency 	<ul style="list-style-type: none"> • Effective for killing spores in suspension, but much longer times required for moist or dry surfaces
Hydrogen Peroxide	<ul style="list-style-type: none"> • Compatible with anionic surfactants • Nontoxic at use concentrations • Does not damage wood or painted surfaces, plastics, rubber or metal • Does not leave residues 	<ul style="list-style-type: none"> • Inactivated by organic matter • Slow acting • No residual killing action 	<ul style="list-style-type: none"> • Excellent (3 to 5%) 	<ul style="list-style-type: none"> • Excellent 	<ul style="list-style-type: none"> • Excellent (6 to 15%)

TABLE 8. — *Comparison of Selected Bactericides (Concluded)*

Disinfectant	Advantages	Disadvantages	Bactericidal Efficiency	Fungicidal Efficiency	Sporicidal Efficiency
Quaternary Ammonium	<ul style="list-style-type: none"> • Colorless, odorless • Nontoxic • Highly stable 	<ul style="list-style-type: none"> • Effectiveness influenced by the chemistry of the water with which it is mixed; colloids, magnesium and iron interfere with action • Layer of soap interferes with action; therefore, soap must first be rinsed away 	<ul style="list-style-type: none"> • Ordinary concentrations do not destroy tubercle bacilli pseudomonas, and other gram negative bacilli 	<ul style="list-style-type: none"> • Not Effective 	<ul style="list-style-type: none"> • Not Effective
Chlorine	<ul style="list-style-type: none"> • Penetrates well 	<ul style="list-style-type: none"> • Increase in alkalinity decreases bactericidal property • Irritating to tissue • Corrodes metal • Injurious to rubber 	<ul style="list-style-type: none"> • Germicidal effect upon bacteria and viruses • Acid-fast bacteria, such as tubercle bacilli, are not destroyed 	<ul style="list-style-type: none"> • Effective 	<ul style="list-style-type: none"> • Effective against spores only if solution is neutral or slightly acid
Formalin	<ul style="list-style-type: none"> • Does not corrode metal • Effective in presence of organic material 	<ul style="list-style-type: none"> • Toxic-irritating fumes • Leaves residuals • Pungent odors 	<ul style="list-style-type: none"> • Very effective 	<ul style="list-style-type: none"> • Effective 	<ul style="list-style-type: none"> • Effective

residues. It also was reported that the hydrogen peroxide-surfactant solution did not observably degrade painted or unpainted wood surfaces, plastic or polymer materials, dense rubber, or a number of metals (ref. 108). In view of these test results, a hydrogen peroxide-surfactant solution appears to be suited as an all-purpose decontaminant for equipment and for room surfaces.

However, as with all bactericides, it needs to be evaluated for each specific application. The solution appears to be a stronger bactericide than the alcohols; it does not leave residues; it is relatively nontoxic to humans at the concentrations proposed for use; and reportedly it causes little material damage. Only three disadvantages are evident: (1) it is not rapid acting; (2) it has no residual killing action; and (3) the solution can be inactivated by organic materials. However, depending on the application, these drawbacks may not outweigh the advantages, and use of hydrogen peroxide-surfactant solutions certainly should receive wider attention.

In addition to their work with hydrogen peroxide, the Russians have also done some recent research in their space program using a solution of beta-propiolactone as a sterilant (ref. 109). Their studies have shown that a 2 percent solution of beta-propiolactone will kill some spores in 40 minutes at 20°C and in 10 minutes at 40°C. The killing power of this solution is temperature dependent, with the sporicidal activity ranging from 9 hours at 0°C to 2 minutes at 60°C.

Along with their work with hydrogen-peroxide and beta-propiolactone, the Russians have been investigating the use of different iodine compounds as disinfectants/sterilants. Soviet workers have obtained three iodophores which they recommend for disinfection: iodopyrron—a complex compound of iodine with polyvinyl-tyrrolidon; iodolan—a complex compound of iodine with hydroxyethyl-lanolin; and iodonate—a complex compound of iodine with sulfonate. All of these are liquids with a dark brown color and without the smell of iodine. They are readily miscible with water and are highly active against vegetative forms of microorganisms, causing death in concentrations of 0.0005-0.001 percent free iodine in 5 to 10 minutes. Iodonate possesses considerable sporicidal powers, causing the death of some spores within 30 minutes (ref. 110). These iodophores possess high activity not only in the wet treatment of surfaces, but also when they are used in the form of aerosols.

WATER PURIFICATION

NASA's research on surface disinfectants led to a number of candidate bactericides, including silver ions, chlorine compounds, and iodine compounds, to keep spacecraft water systems safe from microbial contamination. Chlorines were selected for use in the Apollo Command Module and iodines were used in the Lunar Module. Chlorines were not used in the Lunar Module because they interfered with the operation of the sublimators (instruments used for heat exchange by evaporation of water) by forming chlorohydrates. Although chlorine compounds and iodine compounds are highly effective bactericides, they are corrosive on metals and do not possess sufficient residual killing action. (To be effective for long durations, they must be frequently replenished.) Silver is less corrosive on spacecraft materials, such as aluminum, and has a strong residual killing action; therefore, NASA studied the potential use of silver ions for purifying water.

Numerous investigations have been made on the use of silver and other heavy metals in making water potable, in skin ointments, in antiseptic bandage material, in antibacterial soaps, and for many other bactericidal applications; however, the oligodynamic action of silver is not understood. Even the most biologically active form of silver has not been defined; it may be a positively charged ion, a complex ion, a salt, or some other form (ref. 111). However, silver ions are effective bactericides, are nontoxic to humans in microgram quantities, act fairly rapidly, and have a strong residual effect (i.e., the solutions are biologically active for long periods). On the other hand, this bactericide is more expensive than chlorine or iodine and can be inactivated by organic matter.

A NASA study was intended to determine whether and to what extent silver ions could kill selected bacteria and viruses in very pure water (ref. 112). The bacteria tested were *Achromobacter*, *Alcaligenes*, *Flavobacterium*, and *Pseudomonas*. The results indicated that each genus could be killed by silver in concentrations of 50 and 250 parts per billion (added either as the propionate salt or by electrolysis of elemental silver). The rate of kill varied, depending on cell numbers, the silver concentration, and the temperature of the water. At 120 minutes and at room temperature, silver levels of 50 to 250 ppb killed from 95 to 99.99 percent of the population. The viruses tested were coxsackie, influenza A

(PR8), reovirus (type 1), rhinovirus (type 1A), and vaccinia. The lipid containing viruses (influenza A and vaccinia) were largely insensitive to the silver solutions. The enterovirus was moderately sensitive, and the reovirus and rhinovirus were rapidly inactivated. It was concluded from this study that silver ions could be used for future spacecraft water systems if the concentration of organic matter in the water is low. As a result, silver ions from silver chloride are being considered for use on future spaceshuttles to maintain water potability.

In continuing the feasibility studies of using silver ions for water decontamination, the bactericidal effectiveness of silver in fuel-cell water was evaluated (ref. 113). Silver ions were added by passing water through columns containing silver chloride and silver bromide. The treated water was then challenged by two types of bacteria (type 111A or *Pseudomonas aeruginosa* at $3 \pm 1 \times 10^9$ cells) each day for 7 days. Results showed that total kill was accomplished in a minimum exposure time of 15 minutes at a silver dose of 70 to 100 ppb; however, silver was ineffective against *B. subtilis* spores. Tests are continuing, in order to broaden the effectiveness of this technique to treat a wider range of microorganisms by coupling it to other physical methods, such as heat.

An immediate application suggested by the results of this study is in inhalation therapy equipment. A common problem in the use of the equipment is contamination of the water reservoir by *Pseudomonas*, which can aerosolize and cause severe infections in the patient. Caution has to be exercised in attempting to control the growth of such microorganisms in the reservoir by bactericides, because any bactericide with moderate or high vapor pressure would accompany the evaporated water and could be toxic to the patient. Silver has the advantage of a low vapor pressure and would remain in the reservoir. Thus, the risk of infection can be eliminated and the toxicity hazard from the bactericide can be avoided.

SYNERGISM IN STERILIZATION

The various sterilizing agents have well known limitations and are reasonably well understood. In recent years NASA researchers have tried to combine some of these agents to discover any positive synergistic effects.

If two sterilizing agents exhibit some synergism, it means that the lethal effect of the combined agents is greater than the sum of the effects of the two agents acting separately. A synergistic effect is observed when combining chemical agents (chlorine, formalin, hydrogen peroxide, benzalkonium chloride, etc.) with high-intensity ultrasounds. This is particularly evident in the work of Elpiner, who irradiated hard-to-destroy tuberculosis bacilli, and showed that with ultrasonics it was possible to drastically reduce the dose of chlorine for a 100 percent kill (ref. 114).

In the last few years much research has been done with combinations of chemical disinfectants. Most work has been confined to gas mixtures; e.g., ethylene oxide and methyl bromide, and gases used in conjunction with physical techniques; e.g., ethylene oxide-ultrasonic. There has also been some research using different chemical mixtures and the resulting antimicrobial action.

Work on the Viking prelaunch sterilization project used the principle of synergism with different chemical mixtures. The Viking Lander radioisotope thermoelectric generators (RTG's) had to be provided with a cooling system; and due to planetary quarantine constraints and the possibility of contamination, this cooling system had to be sterilized. The Lander RTG's had to be provided with cooling water whenever the Lander capsule was assembled. Therefore, during sterilization and up to approximately 7 days before launch, clean de-ionized water was circulated through this system and some organisms could have been introduced.

A chemical sterilant had to be found that could be circulated through the system without harming any of the hardware. A sporicide solution was planned to replace the water approximately 7 days before launch and to remain until minutes before launch at which time it would be purged by hot, sterile nitrogen. During the RTG sporicide testing program many chemicals were tested. Different compounds of iodophors, hydrogen peroxide, glutaraldehyde, mixtures of formalin, isopropanol, hexachlorophene, hydrogen peroxide, and Tween 80 were tested for their ability to sterilize the cooling loop. After testing, it was determined that the sporicide solution would be a 70 percent isopropyl alcohol, 8 percent formaldehyde and 22 percent dimineralized water. It was observed that this solution showed the highest activity (5 logs reduction after 48 hours exposure at 55°C) (ref. 115).

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DEVELOPMENT OF SELF-STERILIZING COATINGS AND ENCAPSULANTS

In addition to the discovery of the synergistic effects of physical and chemical agents and the advancements in disinfection mentioned previously, NASA-supported research also led to the development of self-sterilizing coatings and encapsulants by the successful incorporation of bactericides into polymer coatings. The objective was to achieve destruction of microorganisms encapsulated or buried within polymers, such as potting compounds, and to make such compounds self-sterilizing. The harshness of the final sterilization cycle could then be reduced, since microorganisms exposed to the coating or the encapsulant (including those buried or hidden at a surface interface) would have been previously destroyed.

The first trials used formaldehyde (3.7 percent by volume) incorporated into a potassium silicate binder and aluminum silicate pigment (ref. 116). The paint-sterilant mixture was tested by depositing *B. subtilis* spores (1×10^7) on sterile metal coupons and overlaying with the mixture. The samples were then analyzed at weekly intervals (up to four weeks). These preliminary tests indicated that in most cases the total spore population was killed.

After this study, NASA undertook a more comprehensive evaluation. Becton, Dickinson and Company, under the direction of the Marshall Space Flight Center, proceeded with the evaluation and found that paraformaldehyde was more effective as a sterilizing agent than were several other formaldehyde resins tested (ref. 117). With 5 percent paraformaldehyde in the potting compound (RTV), 1×10^5 *B. subtilis* spores encapsulated in the compound were inactivated after 24 hours at 45°C, 2 hours at 60°C, and 15 minutes at 90°C. The inactivation was probably caused by the vaporization of monomeric formaldehyde gas. It was also found that the potting compound-sterilant mixtures were inhibitory against both gram-positive and gram-negative microorganisms. The degree of inactivation was a function of the test organisms, sterilant (type of formaldehyde), and the sterilant concentration.

In addition to the work with formaldehyde, another bactericide was investigated to provide self-sterilizing properties of polymer coatings (ref. 118). An ethylene oxide-dimethylsulfoxide (ETO-DMSO) sporicide mixture was incorporated into several different epoxy formulas at a ratio of 1 part sporicide

to 9 parts resin. A total of 5.4×10^5 *B. subtilis* spores was added to the mixture and then the epoxy was cured. (Curing is the treatment of a material with heat and/or pressure or vacuum, until the material has reached its most stable state.) The amount of reduction varies with the nature of the epoxy resin; but in one case, a 30-minute curing time resulted in a 99 percent destruction of the microorganisms. Testing of the material properties of the epoxies after curing indicated that some were unaffected, but others exhibited increased fragility.

In other studies sponsored by NASA, it was found that some ingredients used in the preparation of potting compounds, conformal coatings, and other encapsulants were, in fact, bactericidal or bacteriostatic in themselves (refs. 119 and 120). In studies on polymer coating made by Pappas, Hsiao, and Hill at North Dakota State University, it was demonstrated that several polymer solvents (e.g., Dow Corning DC801, 802-808, and 840 GE-SR112) show some antimicrobial ability. Initial investigations were conducted using strains of *B. subtilis* to test their ability to survive during the curing process. The initial experiments with spore viability showed that these polymer solvents (especially the amino solvent) act primarily as bacteriostatic agents and not as bactericidal agents (ref. 121). They demonstrated that spores remain viable during curing of a silicone potting compound when n-butylamine is used as a solvent. This bacteriostatic property apparently derives from it being a quite highly volatile compound that evaporates rapidly. Other less volatile amino solvents do not evaporate as rapidly and will reduce the recovery of spores from such potting compounds as a result of their killing action on the vegetative cell as they germinate over the curing time (ref. 122).

Russian scientists apparently have been working along similar but not identical lines. They have found that a number of common bactericides can be incorporated into fabrics and impart their microbiocidal properties to the fabrics. The compounds they have tested include silver, pentachlorophenol, and hexachlorophene. The bactericide preparations are chemically bonded to the fabric rather than being impregnated. The antimicrobial activity was reported as effective against *Staphylococcus aureus*, *E. coli* and anthrax spores. The activity was reported to remain completely effective or was only slightly reduced after 20 to 50 washings (ref. 123).

NEW USES FOR OLD COMPOUNDS

While exploring new techniques in the field of disinfection, NASA also developed new cleansing methods from old compounds.

One such method involves using a formalin solution that does not have the characteristic irritating vapors usually associated with formaldehyde compounds. Sandia Laboratories, in conjunction with NASA research on methods of sterilizing interplanetary spacecraft, developed three new formaldehyde disinfectants that do not emit the irritating odor and vapor of regular formaldehyde. These formaldehyde solutions, developed by R. E. Trujillo and K. F. Lindell, are made by dissolving paraformaldehyde in glycerol, ethylene glycol, or propylene glycol, and relaxing until a clear solution is obtained (ref. 124). The resulting solution is diluted with water 1:100 for normal use. The new solutions are either odorless or have only a slightly musty odor.

Laboratory tests have demonstrated that these new formaldehyde solutions are as effective antimicrobial agents against vegetative cells and spores as regular formalin solutions and that they retain their antimicrobial properties after 40 days of storage (ref. 124).

Recent work has also been done with other older disinfectants that provides new evidence of their ability to kill microorganisms and of their relative effectiveness as spacecraft sterilants. This work has been done with such compounds as dimethyl sulfoxide (DMSO), hydrogen peroxide, peracetic acid,

and sodium hypochlorite (refs. 125, 126, 127, and 128).

APPLICATIONS

Many of the techniques for disinfection mentioned in this chapter have direct application to nonspace industries that have suffered tremendous economic losses from material deterioration. For example, the deterioration of materials through microbiological attack has been a major problem confronting many technical disciplines. Deterioration of painted surfaces from fungi, such as *Pularia pululans*, is a great concern to paint manufacturers. Every year the building industry suffers economic losses from deterioration of polyvinyl chloride and polyurethane building materials.

Another common problem is the deterioration by a fungus (*Alternaria*) of polyurethane diaphragms used in air-conditioning systems. The military has had major problems with deterioration and contamination of electrical equipment coatings, aircraft fuel, and caulking compounds (ref. 129). This list could be greatly expanded.

Now that the concept of incorporating bactericides into paints, polymeric materials, and fabrics has been shown to be feasible, its application should reduce these losses significantly. Furthermore, the medical field stands to benefit since antimicrobial fabrics could be used for such items as hospital bedding and linens, bandage materials, hospital personnel clothing, and the like. This application could possibly reduce the incidence of hospital-borne infection and thereby reduce the cost of health care.

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CHAPTER 5

Gaseous Sterilization

"In sulphurated hydrogen they steeped its wiggly ears"

INTRODUCTION

Gaseous sterilization is the treatment of objects or materials with a chemical in the gaseous or vapor state to destroy all contaminating microorganisms (ref. 130). The most common gases and vapors used are ethylene oxide (ETO), beta-propiolactone (BPL), formaldehyde, methyl bromide (MeBr), propylene oxide (PPO), ozone (O_3), and peracetic acid. Sterilization and decontamination with these gases has been successful in the fields of medicine, food preservation, agriculture, and pharmaceutical manufacturing.

The early beginnings of gaseous sterilization can be traced to Biblical times (antedating the science of bacteriology by hundreds of years) when the concept of purification by burning incense originated (ref. 131). During plague epidemics in the Middle Ages, letters and messages were held in smoke, in the hope that this would prevent the spread of infection. Spices were used not only for flavor, but also because of the belief that the pungent odors reduced food spoilage.

The first instance of gaseous decontamination of air was performed by Lister in 1865 when he sprayed phenol in operating rooms. The use of gases to decontaminate surfaces was greatly assisted by the discovery that formaldehyde, a relatively nondamaging and noncorrosive compound, was bactericidal. Formaldehyde began to be widely used for disinfection in the latter part of the nineteenth century, and its use initiated the concept of gaseous sterilization. Now gaseous sterilization is largely confined to hospital and industrial use.

Gas treatment of surfaces and porous materials offered NASA a potential technique for sterilizing spacecraft at moderate temperatures ($20^\circ C$ or above) and moderate relative humidities (30 to 50 percent). The two principal developments emerging from NASA efforts were: (1) the development of a sterilization process using ETO; and (2) the development of a neutralization technique for formaldehyde.

In addition, an extensive program was carried out to develop parts and materials compatible with these new techniques. This program is discussed in chapter 6.

SELECTING A GASEOUS DECONTAMINANT

The first step in decontamination is to define the restrictions imposed by the application. The factors to consider in gaseous sterilization are:

- The decontamination method must not endanger the well-being of people in the nearby vicinity.
- The treatment must be reliable so that the chance of severe or fatal infection is reduced to the absolute minimum.
- The room must be decontaminated in the shortest time possible to reduce laboratory downtime.
- The decontamination method must not damage the centrifuges, incubators, refrigerators, freezers, water baths, and other equipment in the room.
- The relative humidity of the room can be raised as much as 70 to 80 percent by placing vaporizers throughout the area.
- The temperature of the room can be raised about $10^\circ C$ by using a series of portable heaters.
- The room must be sealed in a fast and simple manner by use of an inexpensive sealant, or by taping plastic sheeting over cracks, doors, and windows.

Now that the restrictions of the application have been defined, the alternative gases need to be considered. Comparing the restrictive characteristics of the gases presented in table 9, a sterilization process can be selected. In this case ETO would be eliminated as a candidate for three reasons. First, ETO takes too long to decontaminate. Second, ETO is such a strong penetrant, that the room could not effectively be

TABLE 9. — *Characteristics of Commonly Used Gaseous Agents*

Selection Parameters	Gaseous Sterilant						
	Ethylene Oxide	Formaldehyde	Beta Propiolactone	Methyl Bromide	Propylene Oxide	Ozone	Peracetic Acid
General Conditions for Use:							
Relative humidity, percent	30 to 50	60 to 80 ¹	75	30 to 50	25 to 50	60 to 80	40 to 80
Temperature range, °C	25 to 60	20 to 60	-10 to 27	25 to 60	25 to 60	0 to 20	25°C/Optimum
Gas Concentration, mg/l	100-300	1-15	2-5	3500	800-2000	0.001 to 5	1-5
Time, hr.	Slow (8 to 16)	Normally slow dependent upon protective coating, 0.5 to 10	Fast, 0.5 to 2	Slow (8 to 16)	Slow (8 to 16)	Dependent upon application	Fast, 0.33 to 1.33
Bactericidal Activity	Effective against all organisms	Effective against all organisms	Effective against all organisms	Effective against all organisms	Effective against all organisms	Varies with type of organism	Effective against all organisms
Penetrability	Strong	Weak	Weak	Strong	Moderate	Strong	Weak
Material Compatibility	Minimal effects	No direct damage but residual polymers are a problem	No damage from vapor, but the condensate is difficult to remove	Relatively little damage	Minimal effects	Strong oxidant	No damage from vapor at dilute concentrations, liquid is corrosive
Equipment and Apparatus	Wide range of commercially available chambers or containers	Easily disseminated by vaporizers, household appliances or a chemical reaction.	Lack of available disseminators to produce aerosol droplets small enough to vaporize before they settle out	Easily released from gas pressure vessels. Can also be used in same equipment as ETO	Easily disseminated by vaporizers, etc.	Released from gas pressure vessels but high concentrations are difficult to maintain.	Easily disseminated by vaporizers, etc.

¹ 60-80% is optimal, but formaldehyde generated from paraformaldehyde can be successfully employed at humidities as low as 30%.

TABLE 9. - *Characteristics of Commonly Used Gaseous Agents (Concluded)*

Gaseous Sterilant							
Selection Parameters	Ethylene Oxide	Formaldehyde	Beta Propiolactone	Methyl Bromide	Propylene Oxide	Ozone	Peracetic Acid
Residues	None are normally encountered, can form ethylene chlorohydrin with chlorine containing plastics or if salt is present	Residues are formed by polymerization of the vapor on surfaces but can be neutralized under certain conditions	No residues from the vapor, but the liquid condensate is difficult to remove	None	None normally encountered, can form propylene chlorohydrin, with chlorine containing plastics or if salt is present	None	Decomposition products are acetic acid, water, and oxygen
Hazards							
Human Toxicity	Moderate (50ppb max), liquid or aqueous solutions have vesicant properties. Ethylene chlorohydrin is toxic when ingested	Pungent qualities prevent over exposure	Gas tolerance is unknown. Liquid is strong vesicant, and is a carcinogen for rats and mice	Moderate, 20ppm is threshold value for man	Vapor threshold for man is 0.24 mg/l. Liquid and aqueous solution have vesicant properties	Toxic, 0.001 mg/l is the limit tolerable for man	Pungent qualities prevent over-exposure decomposition products are oxygen and acetic acid
Flammability	Requires dilution with fluorinated hydrocarbons, CO ₂ , MeBr, etc.	Not flammable in dilute concentrations	Not flammable in dilute concentrations	Not flammable	Can be diluted to reduce hazard as with ETO	Not flammable	Not flammable in dilute concentrations
Common Applications	Heat and moisture sensitive materials such as disposable pharmaceuticals, medical devices, and implements, chemical and biological solutions	Decontamination of room enclosures, imported wool and hair, and veterinary applications	Decontamination of room enclosures, imported wool and hair and veterinary applications	Spacecraft sterilization in conjunction with ethylene oxide	Food preservation, decontamination of spices, powders and flaked foods	Decontamination of swimming pool water, food preservation by prevention of mold surface growth	Decontamination of room enclosures and gnotobiological research

sealed off to prevent the gas from escaping, thus making it difficult to maintain a lethally effective concentration. Third, the escaped gas is toxic to humans and would be a hazard to personnel in the vicinity. PPO is ruled out for the same reasons.

MeBr would be similarly discarded because it is even harder to confine than ETO. The concentration (3500 mg/l) required for MeBr to be an effective bactericide cannot be maintained because the gas escapes so readily. Moreover, the gas would be a hazard to nearby personnel, for the toxicity threshold value for man is 20 ppm.

Ozone is unsuitable because it is toxic to humans and would escape from the room. It might also damage the equipment in the room, especially rubber items.

The only three gases (actually vapors) remaining are BPL, formaldehyde, and peracetic acid. All three are highly effective bactericides and they can be used at ambient temperatures. The toxicity of formaldehyde and peracetic acid is not a problem, since the gases are easily confined by a simply sealing procedure (taping plastic over cracks and openings). They are more effective at relative humidities of 60 to 80 percent, which can be achieved with a series of vaporizers. Raising the temperature of the room 5°C to 10°C with portable heaters would increase the rate of kill and reduce the time necessary for decontamination.

Neither formaldehyde nor BPL vapor will damage the equipment or room surfaces. Although formaldehyde vapor is not corrosive, it does leave residues after treatment that are difficult to remove. This problem can be partially circumvented by neutralizing the vapor with ammonia gas, although this procedure has not always been satisfactory. With proper application, an innocuous white powder is formed which slowly decomposes into formaldehyde and ammonia. The chief advantage of this procedure is that with adequate ventilation the room becomes odorless and usable shortly after neutralization. Peracetic acid vapor is corrosive, but not at the concentrations normally employed for decontamination.

Liquid BPL is corrosive and the apparatus used for its dissemination has not been perfected. Highly trained personnel are required to administer BPL vapor to prevent the formation of liquid condensates. More important, BPL has been implicated as a potential carcinogen and its use by the general public for surface decontamination is restricted by the

Environmental Protection Agency. Based on the previous considerations, the selection of the gaseous agent can now be made. BPL is ruled out because it is difficult to administer and its use is restricted by government regulations. Peracetic acid would be selected if direct ventilation were possible. If ventilation were not possible, formaldehyde with neutralization could be employed.

In many cases, the selection of a sterilizing gas depends on the type of conditions required to achieve sterilization. Once the sensitivity of the items to be sterilized is determined, tradeoffs in conditions can be made to reduce potential damage and ensure sterilization. For example, in the ETO sterilization of medical equipment, the product may be more sensitive to breakdown by heat than by moisture. From the ranges indicated in table 9, a low temperature (25°C) could be used in combination with maximum humidity values and gas concentration.

GENERAL PROPERTIES OF ETHYLENE OXIDE (ETO)

Proper application of gaseous sterilization requires more indepth understanding of the nature of the gases used and the individual parameters that affect their performance.

The biological properties of ETO were first noticed in 1928 when the gas was effectively used as an insecticide. After this discovery, its first general use was as a preservative for foods and spices. Its potential for sterilizing heat- and moisture-sensitive products was established by a group of researchers at the U.S. Army Chemical Corps, Fort Detrick, Maryland. It is now widely used as a sterilant in the pharmaceutical and medical supply industries (ref. 132).

Chemical Properties

ETO, CH_2OCH_2 , is a colorless gas at room temperature, boils at 10.8°C and freezes at -111.3°C. As a liquid, it is soluble in water in all concentrations and in solids such as rubber, leather, and plastic (ref. 133). ETO is highly flammable and may explode when mixed with air in concentrations between 3 and 80 percent by volume. To limit this hazard, it is generally used with a carrier or diluent gas; the most common mixture is 12 percent ETO and 88 percent Freon 12.

Biological Effects

The biological effects of a gas refer to the extent of kill of microorganisms under specified conditions. The conditions which can modify the extent of kill are temperature, gas concentration, relative humidity, and time of exposure.

Temperature.—ETO can be used as a sterilant over the temperature range of 20°C to 60°C. Elevating the temperature in this range reduces the time required for exposure (ref. 134). The ability to sterilize at a moderate temperature, such as 20°C, represents a major advantage of gas sterilization in general. At 54°C, the kill rate over a wide range of concentrations is probably limited only by penetrability, and therefore, ETO is recommended for general applications (ref. 130). With commercial equipment designed mainly for hospital use, temperatures in the range of 50°C to 60°C are usually employed, but ETO provides the flexibility of treating products at lower temperatures if material degradation is a problem.

Gas Concentration.—ETO gas concentrations are expressed as milligrams per liter (mg/l), which is equivalent for practical purposes to ounces per 1000 cubic feet of chamber space. With a lower sterilizing temperature, a higher gas concentration increases the biological effect and reduces the time required for sterilization. Reducing the concentration by half (from 880 mg/l to 440 mg/l) doubles the time required for sterilization. The lethal effect of ETO is dependent on the amount of ETO in a given volume of space and is not related to the amounts of other diluent compounds present (ref. 135).

Gas concentration can be determined by any of several methods. Partial pressure measurements and chemical analysis of the chamber atmosphere were methods formerly used. Now, nondispersed infrared spectroscopy and gas chromatography (ref. 133) are usually preferred because they are quicker and much more sensitive methods.

Relative Humidity.—The effect of moisture on the ETO sterilization process is complex and its interaction at the microorganism level is not completely understood. There are conflicting reports, but it is generally accepted that humidity in the range of 30 to 60 percent creates the most desirable effects. Recent studies (ref. 136) indicate that a relative humidity of 50 percent is more effective than a RH of 30 percent (50°C, ETO concentration of 800 mg/l). When relative humidities are increased to 75 to 85 percent, the biological effect is significantly reduced.

Microorganisms dried beyond a critical point do not react uniformly to ETO sterilization (refs. 135 and 137). The increased resistance resulting from dehydration can be significant and should be considered when a sterilization cycle is being established. The problem can be rapidly overcome by directly moistening the product to rehydrate the contaminants. In most cases, the nature of the product precludes this solution, so that prehumidification at an elevated relative humidity is necessary. Although rehydration is time consuming and less convenient, it prevents degradation of the product.

Exposure Time.—Exposure time is based on relative humidity, temperature, gas concentration, degree and type of contamination, type of materials, and volume of the chamber load. ETO sterilization is not a rapid process and in selecting conditions for delicate materials, a 12-hour cycle is not uncommon. It is advantageous to derive minimum sterilization process times, rather than apply overkill conditions that may cause product degradation. This is accomplished by performing test runs, determining the representative death rate, and linearly extrapolating the desired probability of sterilization at certain exposure times.

Microorganism Susceptibility to ETO

Apparently, all microorganisms are susceptible to the effects of ETO. Many bacteria, some of which are *B. subtilis*, *Clostridium sporogenes*, *S. aureus*, *Sarcina lutea*, mycobacteria, *B. anthracis*, hemolytic *Streptococcus*, and fungi are sensitive (ref. 133). In general, spore formers are slightly more resistant (two- to sixfold) than vegetative bacteria, yeasts, or molds. *Staphylococcus* and *Bacillus* species are more resistant than most other genera of bacteria (ref. 130). Some organisms exhibit nonuniform resistance that is related to the lipid (fat) content of the cell (ref. 138). The list of viruses susceptible to ETO include vaccinia, influenza A and B, Newcastle disease virus, Theiler's mouse encephalomyelitis virus, lymphocytic choriomeningitis virus, Eastern equine encephalitis virus, and foot-and-mouth disease virus.

Penetrability

One of the most advantageous characteristics of ETO is its penetrability. The list and types of materials that can be penetrated by ETO include a variety of plastics such as polyethylene, cellulose acetate,

ethylcellulose, nylon film, polypropylene, polyvinyl chloride, and rubber (refs. 130 and 133). The ability of ETO to penetrate wrapping materials permits the sterilization of prepackaged medical and pharmaceutical products and thus reduces the recontamination hazard of post-sterilization packaging. Unreacted gas is usually dissipated by airing following sterilization. Rubber and plastic items, in which ethylene oxide can dissolve, require at least 48 hours of aeration. Also, certain byproducts, such as ethylene chlorohydrin, can be formed which are difficult to remove (ref. 139). Selection of packaging materials is limited because certain plastics, such as polyvinyl alcohol and laminated polyester, are poorly penetrated by ETO. Natural contamination of the products being treated can also pose a problem (ref. 140).

Materials Compatibility

One of the tasks undertaken by NASA was the classification of the effects of ETO on many types of materials. Only a few highlights are presented in this section. Further details are contained in chapter 6.

In general, ethylene oxide gas is relatively non-corrosive and nondamaging, but ETO-fluorocarbon mixtures (discussed in a later section of this chapter) can degrade some material. Both silver and copper can catalyze polymerization of ETO if moisture is available and they may cause an explosive reaction if acetylene contaminants are present in the ETO mixture. Steel or iron containing any trace of rust should not be used with ETO. Certain polymers and plastics absorb ETO gas, but for the most part, there is minimal degradation. More frequently, degradation is associated with the solvent action of Freon-12, but in these cases it can be avoided by using carbon dioxide or methyl bromide as the diluent gas. For spacecraft sterilization, any deterioration can be a serious problem, since the slightest malfunction can ruin the success of a mission. However, for the routine sterilization of pharmaceutical plastics and similar products, ETO is considered nondegrading.

Equipment, Apparatus, and Method of Use

There are many different types of chambers and cabinets used for ETO sterilization. Laboratory containers or chambers (refs. 130, 133, 139, and 141)

range from tightly sealed bags to the commercially available equipment shown in figure 30. These units have humidity, pressure, and temperature controls, several types of online sensors, and can be fully automatic. ETO sterilization is accomplished in five basic cycles. In the prehumidification phase, a vacuum is created in the preheated chamber while moisture is maintained by the addition of a small amount of steam. Next, the ETO mixture is introduced to the desired concentration (partial pressure). A fan or blower is used to circulate the ETO mixture. Exposure begins from this stage, and is completed when a final vacuum removes the ETO gas. In this cycle, the chamber is returned to atmospheric pressure by introducing filtered air.

When conventional ETO sterilizers were used, degradation occurred with a number of materials (such as metal foils) used on spacecraft. This breakdown was attributed to the stratification of water vapor in the chamber, resulting in condensation in certain locations; therefore, NASA sponsored the development of a system which had additional capabilities.

The system, shown in figures 31 through 34, was developed by Becton, Dickinson and Company under the direction of the Jet Propulsion Laboratory (ref. 136). It is capable of exposing test items to a dynamic environment, so that stratification either of ETO or water vapor does not occur in the chamber. The ETO gas mixture is continuously circulated through an outside preheated reservoir into the chamber and back. Moisture is introduced into the gas mixture in the reservoir rather than directly into the chamber as with conventional ETO sterilizers. Slight changes in ETO concentration, temperature, and humidity are rapidly detected by sensitive monitoring devices and adjustments are made to rectify deviations from the desired cycle by altering the conditions of the reservoir operation.

The monitoring system consists of a thermistor for sensing the temperature of the load, a potentiometer-thermocouple set-temperature verification, a Bacharach moisture sensor, an infrared analyzer for ETO concentration, a thermometer for monitoring temperature of the vaporized (ETO-fluorocarbon) mixture, a Dickson Minicorder for monitoring vacuum, pressure, and time during each cycle, and a Bourdon-tube compound gage as a backup.

The primary objective of this program was to develop a sterilization chamber in which material

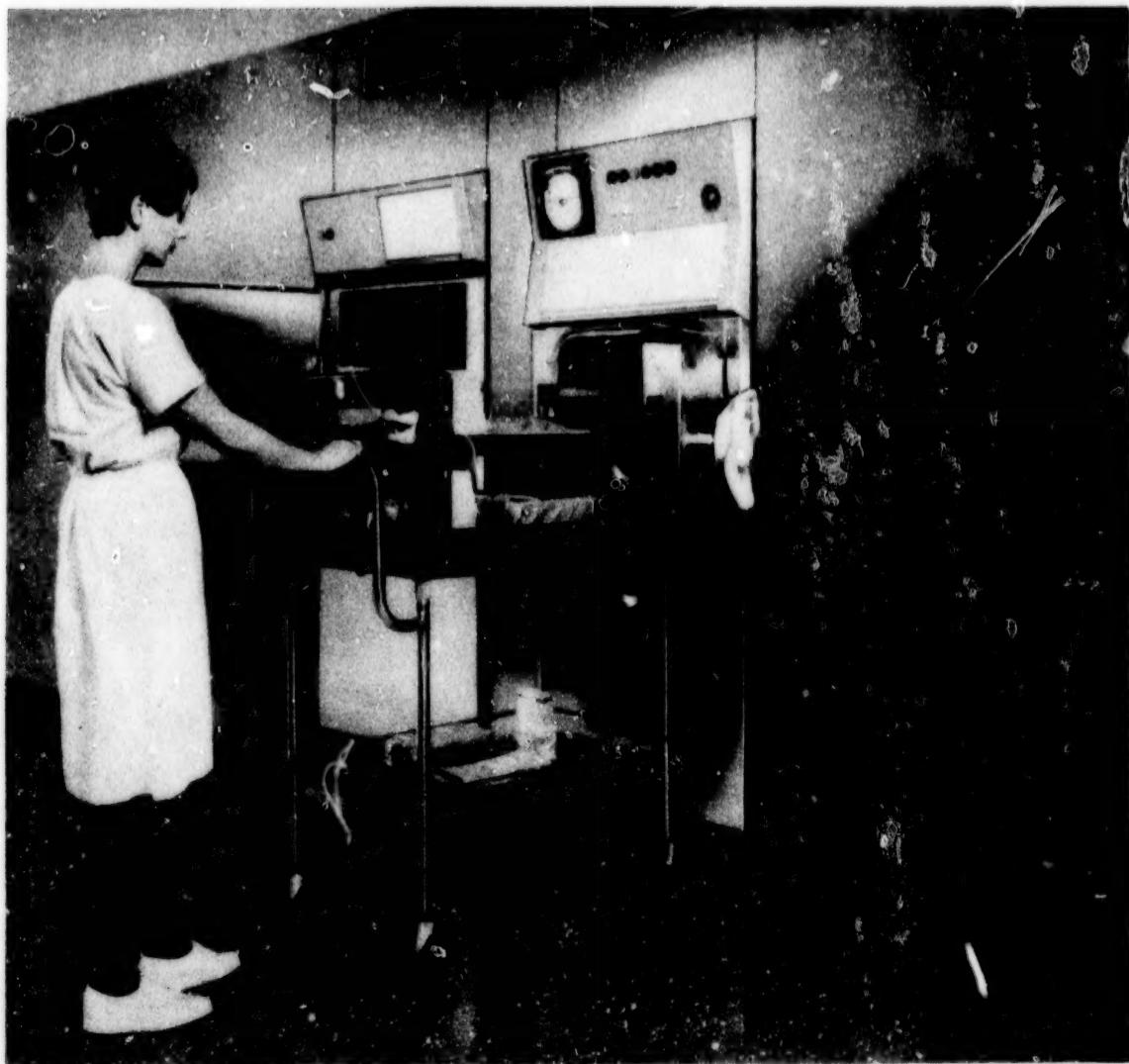


FIGURE 30. — Commercially available ETO sterilizer.

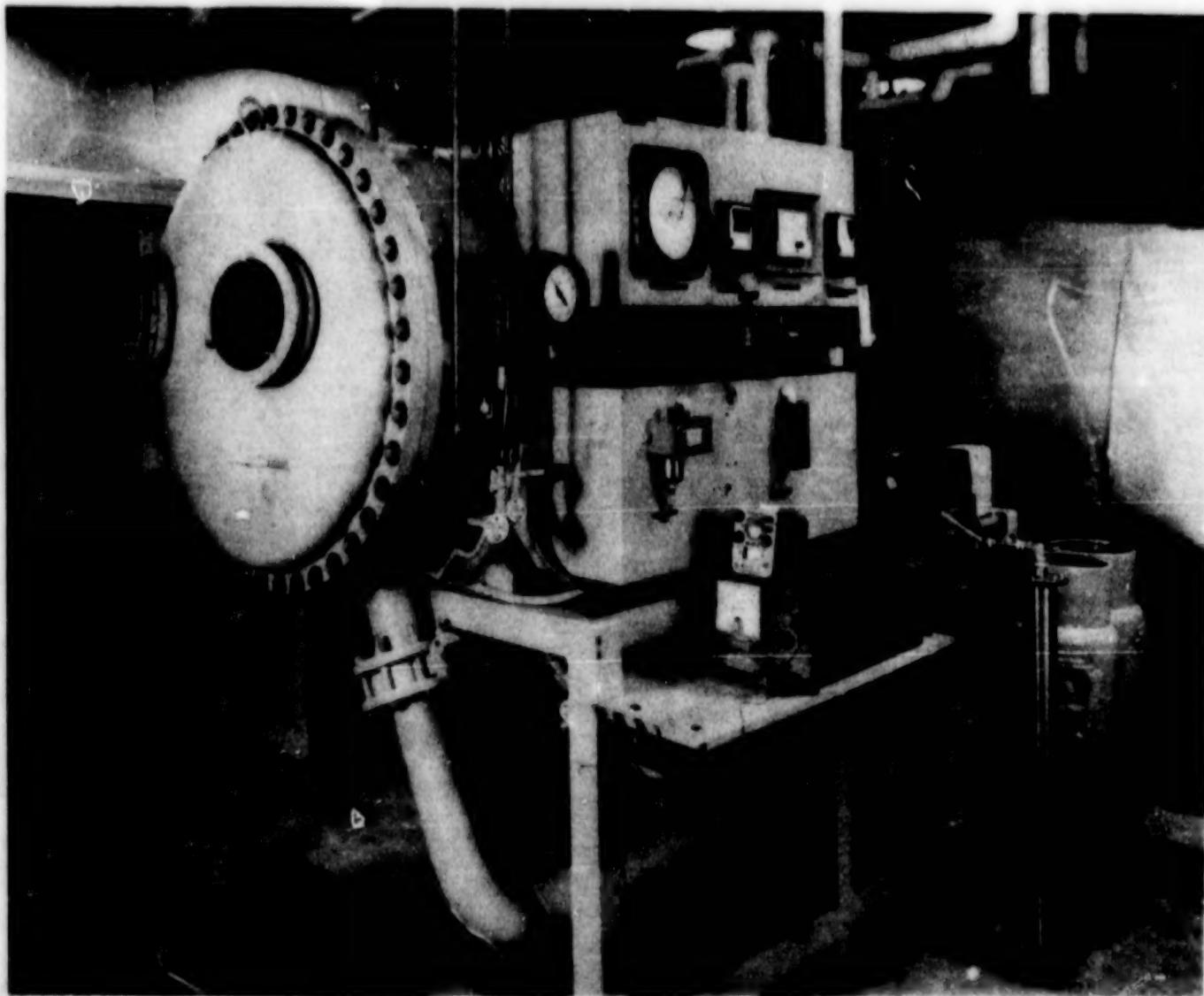


FIGURE 31. - An overall view of the right side of the decontamination chamber showing view ports, instrument panel and ethylene oxide cylinders.

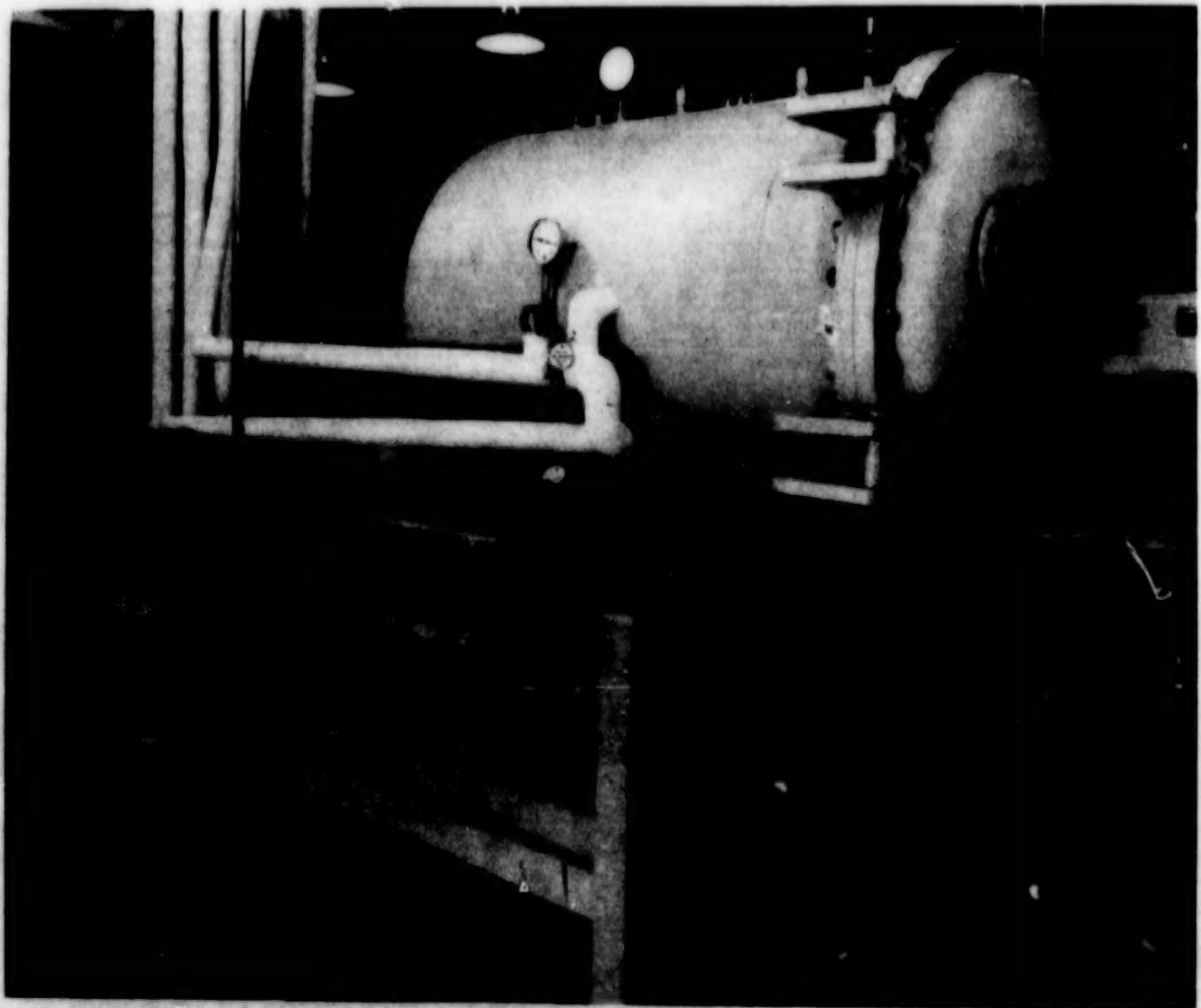


FIGURE 32. — An overall view of the left side of the decontamination chamber showing steam injection pipes, vacuum system, modutrol assembly for main heat exchanger.

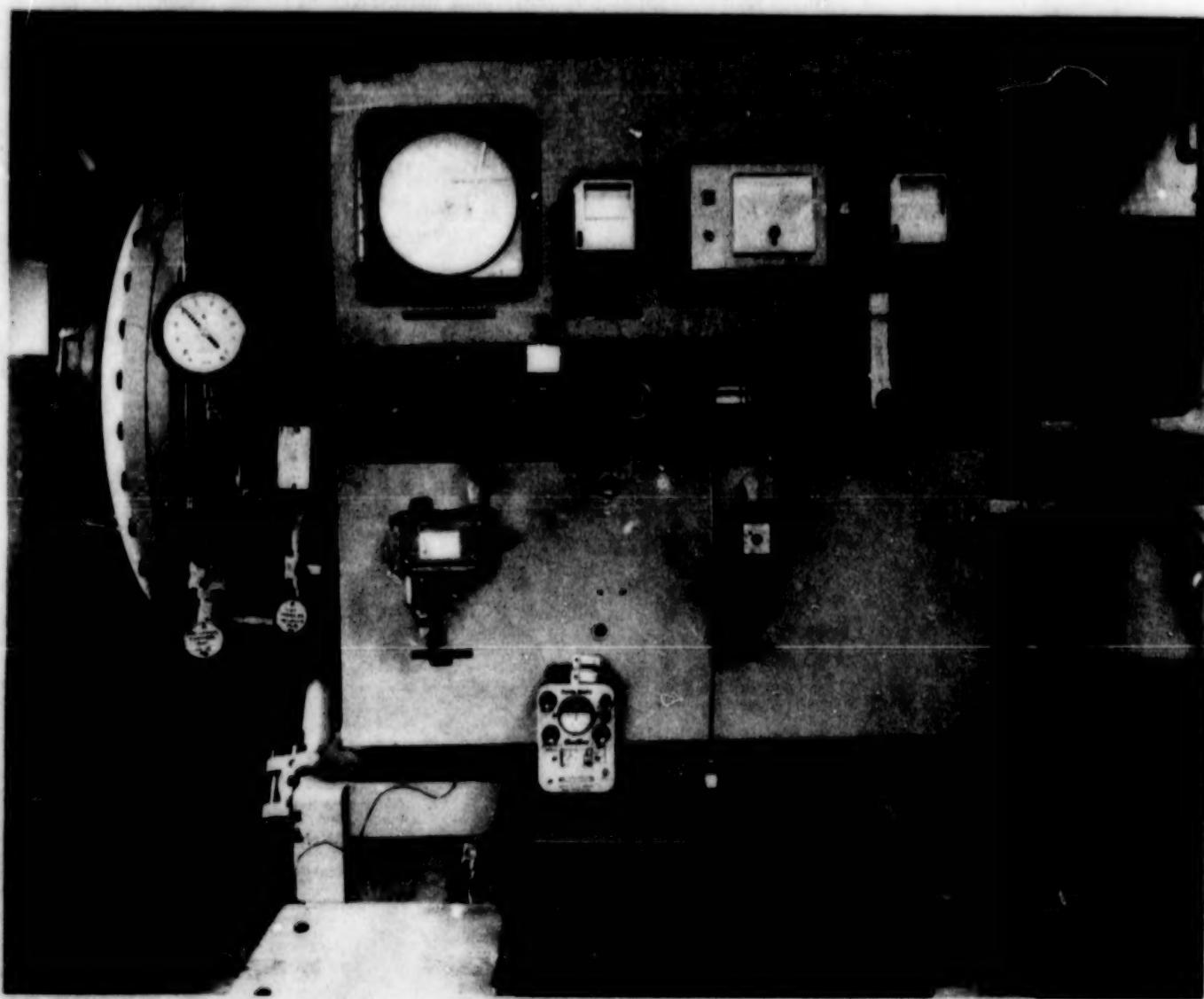


FIGURE 33. — View of instrumentation panel showing temperature and pressure controls and recorders, LIRA, and potentiometer.

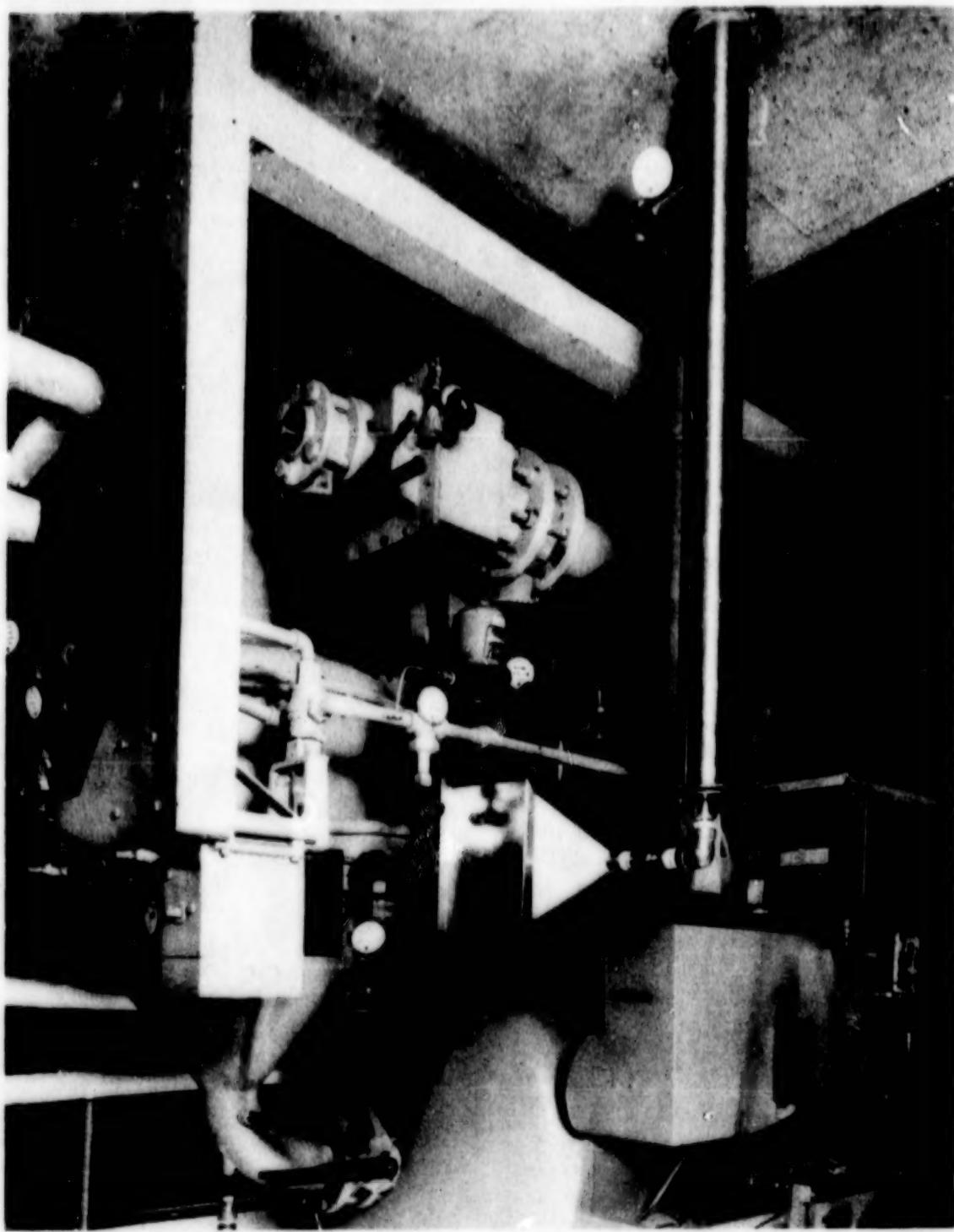


FIGURE 34. — View showing drying column and filter; absolute filter housing and inlet; and ethylene oxide heat exchange and filter.

degradation during ETO sterilization would be minimized. The second objective was to define an optimum ETO sterilization process, considering variation in gas concentration, time, temperature, and humidity. The first goal was achieved. Preliminary results of the Becton, Dickinson study indicated that the most effective ETO sterilization cycle for maximum kill under the conditions set forth was achieved at 50°C, at a relative humidity of 50 percent, with an ETO concentration of 800 mg/l for 24 hours. With modifications, this unit has potential use for the sterilization of sensitive medical equipment.

Advantages and Limitations of ETO Decontamination

In summary, the advantages of ETO sterilization are that effectiveness is provided at room temperature and at moderate humidities, few materials are significantly damaged, few residuals of the gas are left after use with most materials, and the gas has the capability of penetrating many materials.

Some of the drawbacks in ETO sterilization are: the length of time necessary for sterilization, the human toxicity of ETO in the liquid or vapor state, the relatively higher cost, and the flammability problem. The flammability hazard can be avoided by diluting the gas with carbon dioxide (90 percent CO₂ to 10 percent ETO), fluorinated hydrocarbons (88 percent Freon-12 to 12 percent ETO), and MeBr (40 percent ETO to 60 percent MeBr). ETO is a strong vesicant. Treated clothing—especially shoes, rubber articles, and plastic gloves—should be aerated after sterilization to avoid skin irritation. The use of ETO for food application has been forbidden by the 1958 Food Additive Amendment to the Food, Drug, and Cosmetic Act because of the potential hazard of the formation of toxic compounds such as ethylene glycol in the food product. Concern should also be given to ethylene chlorohydrin, which is more toxic. It may be formed when salt is present or with chlorine containing plastics.

Protection (ETO resistance) can be afforded to spores trapped within water-soluble crystals such as salt (ref. 142). Microorganisms buried in layers of dense organic matter, such as dried pus, blood, or serum can also be protected (ref. 130). These resistance factors require longer sterilization times. Finally, longer-than-normal sterilization times (dictated by the need for a lengthened humidification period) are required for products when contaminants have previously been exposed to dry conditions.

Applications of ETO

The list of items that have been successfully sterilized with ETO is extensive (refs. 130 and 143). Some of these are:

- Pharmaceuticals—catgut sutures, papain, plastic equipment parts and bottle closures, antibiotics, plastic bandages, certain plastic disposables, and rubber laboratory items
- Medical instruments and devices—ophthalmic instruments, catheters, syringes, cystoscopes, bronchoscopes, forceps, extractors, scalpel blades and holders, clinical thermometers, and dental and root-canal files
- Equipment—centrifuges, microscopes, heart-lung machines, infant incubators, artificial kidney machines, oxygen tents, and intravenous sets
- Other items—microbiological culture media, pipettes, petri plates, books, hospital bedding, soil, clothing and footwear, vaseline, motor oil, and electric cords.

GENERAL PROPERTIES OF FORMALDEHYDE

Formaldehyde fumigation of contaminated sick rooms was widely used in the early part of the 20th century. Its use was dramatically reduced because of the control of major epidemics by other means and evidence that it was difficult to effectively administer (ref. 139). Now that the use of BPL, once considered as a replacement, is curtailed and easier and improved methods of formaldehyde decontamination are available, its use is increasing.

Chemical Properties

Formaldehyde, a gas at ambient temperatures, is commercially available only as a solid polymer, para-formaldehyde, or as an aqueous solution, formalin, which is approximately 37 percent formaldehyde with some methanol added as a stabilizer.

Earlier, formalin was the most convenient source of formaldehyde gas. The liquid could be boiled or sprayed as a fine mist that would vaporize before settling out. Formaldehyde gas can also be obtained from the chemical reaction between potassium permanganate (KMnO₄) and aqueous formalin, which is highly exothermic (ref. 144).

Paraformaldehyde does not melt when heated, but sublimates to release unpolymerized formaldehyde gas. It is combustible with a flash point of 93°C and an ignition temperature of about 302°C. It is flammable in air over a range of 7 to 73 percent gas per volume of air; but explosions rarely occur at the concentrations used for decontamination. It is difficult to maintain high concentrations in an enclosed space or at high humidities, since formaldehyde tends to absorb and polymerize on exposed surfaces and to dissolve in moisture on these surfaces, polymerizing again when the surfaces dry. Recently, paraformaldehyde has become commercially available in a convenient pellet form, rather than as a fine powder that sublimates much more readily when heated. Because the dry gas produced by the pellet causes fewer residue problems, interest has increased in the use of formaldehyde as a decontaminant since its introduction (ref. 145).

Biological Effects

The lethal effectiveness of formaldehyde gas is modified by temperature, gas concentration, relative humidity, and time.

Temperature.—Formaldehyde vapor is effective as a sterilizing agent at room temperature (ref. 139). The lethal efficiency is stimulated by increasing the temperature from 20°C to 60°C. In practice, however, the most common use of formaldehyde is the terminal disinfection of rooms that are normally sealed off and more conveniently disinfected at ambient temperature.

Gas Concentration.—For the sterilization of enclosed spaces, liquid formalin is vaporized at a rate of 18 to 36 ml/m³ of enclosed space. This distributes a theoretical dosage of 7.5 to 15 mg of formaldehyde per liter of air (ref. 139). Lower concentrations (1 to 5 mg/l) have also achieved sterilization (ref. 138). In nutrient broth (a microbiological growth medium), concentrations as low as 27 mg/ml can maintain sterility and concentrations ranging from 15 to 27 mg/ml are growth inhibitory (ref. 146).

Relative Humidity.—For practical applications, a 70 percent relative humidity is the optimum, but anywhere in the range of 60 to 80 percent RH is suitable. Increased relative humidity causes a greater accumulation of formaldehyde on surfaces, with a maximum accumulated at a relative humidity of 83

percent (ref. 146). Lower humidities (30 percent) can be used, but in this case it appears that formaldehyde vapor generated from paraformaldehyde is more effective than vaporized formalin—possibly because of dilution by condensation of the latter (ref. 147).

Time.—Sterilization times can vary from 30 minutes to 10 hours. Disinfection of contaminated rooms is generally accomplished within a 6-hour exposure period. In addition to the temperature, gas concentration, and relative humidity, the time for sterilization is dependent on the nature and number of contaminating microorganisms, and the presence of any material that absorbs the gas.

Microorganisms Susceptibility

All bacteria, fungi, and viruses, including spores, are killed by formaldehyde gas; however, the extent and rate of kill are dependent on direct exposure of the gas to the microorganisms (ref. 138). Protection afforded by even a thin coating of organic matter can substantially reduce the lethal effects of this gas. Formaldehyde is generally considered bactericidal and not bacteriostatic; however, some investigators have found its effect to be reversible within certain limitations (ref. 138). After exposure to formaldehyde, rapid neutralization with sulphite ions, or with a combination of dimedone and morpholine (0.2 percent) can cause reversal of the lethal effects; however, this process is not yet understood.

Penetrability

Formalin vapor is primarily a surface sterilant and does not readily diffuse into porous materials. For example, tests showed that nonsporeforming microorganisms, buried in a few folds of a blanket, were not affected at all after prolonged exposure (ref. 138). Small amounts of soil can be sterilized only after first subjecting the material to a vacuum and then flashing in the gas.

The penetrability of formaldehyde gas generated from paraformaldehyde and from vaporized formalin was compared in three materials, cotton, glassine, and paper. Tests revealed paraformaldehyde to be more effective at lower humidities (33 percent) (ref. 147). This is partly due to the reduction in the formalin vapor concentration by condensation, at this humidity level. The researchers found no difference in the penetrability of the two formaldehyde sources at

100 percent RH, and concluded that the activity of formaldehyde from the two sources is the same.

Materials Compatibility

Formaldehyde vapors themselves are relatively noncorrosive and nondamaging; however, the high humidities required for effective formaldehyde sterilization from formalin vaporization can cause corrosion of metals. But sophisticated mechanical, electronic, and optical equipment has been sterilized with formaldehyde gas from paraformaldehyde at 30 percent relative humidity with no observable operational loss (ref. 147).

Equipment, Apparatus, and Method of Use

Formaldehyde gas is usually generated in three simple ways. First, formalin can be atomized (18 to 36 ml of formalin per m³ of enclosed space) with various spraying devices. Secondly, paraformaldehyde powder can be vaporized by using common household appliances such as electric frying pans and deep-fat fryers. With a deep-fat fryer, a silicone fluid is mixed with the paraformaldehyde to achieve a uniform and even heat for efficient dispersion. Thirdly, where no electricity is available, formaldehyde can be generated by reacting formalin with potassium permanganate at a ratio of 60 to 40 percent by weight volume. New data indicates that the vaporization of paraformaldehyde is the most effective approach (refs. 144 and 146). The chemical is spread more uniformly and less condensation and polymerization occur within the enclosure. After treatment, the dry gas is eliminated by aeration (ref. 144).

Advantages and Limitations of Formaldehyde Decontamination

The advantages of using odorless, vaporless formaldehyde for decontamination and disinfection are:

1. Decontamination can be accomplished at room temperature
2. The liquid is bactericidal and is highly effective against all organisms tested
- 3. The decontaminant is easy to administer
4. The liquid does not damage materials.

In the past, the limitations of formaldehyde for practical applications were its lack of penetration,

extreme pungency, and difficult-to-remove residual polymers. Currently, the penetration problem can be minimized in certain situations by preevacuation. Formaldehyde's pungent odor formerly required the routine venting of a room after decontamination. This operation could take as little as 1 day, but it generally took 2 to 3 days before work could be resumed. Residual polymers were left on surfaces after the evaporation of water from the vapor and were difficult to remove.

The neutralization of formaldehyde with anhydrous ammonia (discussed in chapter 4) overcomes both the slow ventilation problem and the residual polymer problem in certain situations. The product of the neutralization reaction is a white, odorless powder, hexamethylenetetramine that slowly decomposes to ammonia and formaldehyde. Although the basic technique has been known for a long time, renewed interest was generated through NASA research at the Marshall Space Flight Center. Becton, Dickinson and Company successfully developed a modification of this technique that is safe and easy to use. The process uses anhydrous ammonia gas rather than the aqueous form. In the studies to date, this neutralization has permitted entrance into an area shortly after formaldehyde decontamination.

Applications of Formaldehyde

Formaldehyde fumigation is used to disinfect laboratory rooms, veterinary clinics, and textile mills. Other applications include the sterilization of surgical or medical equipment, pharmaceuticals, and hospital bedding. Certain disease-carrying materials, such as raw wool, from areas where anthrax is present, have been decontaminated by formaldehyde. Poultry houses have been fumigated with formaldehyde to destroy pathogenic fungi such as *Histoplasmosis* or viruses such as Newcastle disease virus. Electo-optical and mechanical equipment have also been sterilized with this gas.

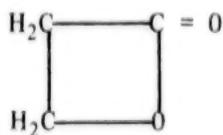
GENERAL PROPERTIES OF BETA-PROPIOLACTONE

Interest in the sterilizing properties of beta-propiolactone (BPL) compound arose in the mid-1950's. Aqueous solutions were used to treat blood plasma in an attempt to reduce the occurrence of infections (such as hepatitis) resulting from

transfusions. Following this effort, the U.S. Army Biological Laboratories started a program to evaluate the effectiveness of BPL in its vapor form. This resulted in the recommendation that it be used as a replacement for those applications then using formaldehyde.

Chemical Properties

BPL structurally resembles ethylene oxide:



It is a colorless liquid at room temperature and decomposes at a boiling point of 162°C. At 25°C the vapor pressure is 3.4 mm Hg, the saturated vapor concentration is 8.1 mg/l, and the solubility in water is 37 percent by volume. The liquid is stable at 4°C for at least 2 years and its half-life in dilute aqueous solutions is approximately 3.5 hours at 25°C (ref. 148). Beta-propiolactone is not flammable in air in concentrations which theoretically can exist under normal atmospheric conditions (ref. 148).

Biological Effects

Temperature.—BPL is effective at relatively low temperatures (-10°C). It is also effective at higher temperatures (up to 25°C) where the exposure time can be decreased.

Gas Concentration.—The usual concentration of BPL for sterilizing purposes is 1 to 5 mg/l in air (ref. 149). The rate of kill is increased at greater concentrations. In terms of concentration of gas required and observed rate of microbial inactivation, BPL is the most effective sterilizing gas.

Gas detection tubes can be used to monitor the concentration of BPL with a sensitivity of 0.002 to 0.004 mg (ref. 150). The test is based on the formation of a blue color, caused by the alkylation of gamma (p-nitrobenzyl) pyridine, absorbed on silica gel inside a small glass tube through which air is drawn. These gas-detection tubes were developed by the U.S. Army Chemical Corps, but similar detection tubes are available commercially (ref. 150).

Relative Humidity.—BPL requires a relative humidity of 75 percent to effect optimum kill (ref. 148). At lower humidities, the rate of kill is diminished. Dried spores require a higher humidity level during gas exposure to achieve the same inactivation.

Time.—Sterilization or decontamination with BPL is rapid. The exposure time recommended (ref. 151) for large enclosed areas (with 2 to 4 mg of BPL/l air, and a relative humidity of 74 percent at 24°C) is 2 hours.

Microorganism Susceptibility

BPL is active against spores, bacteria, rickettsia, and viruses (refs. 130, 148, 152, and 153). Some pathogenic agents that have been treated effectively with BPL are Venezuelan equine encephalomyelitis virus, smallpox, yellow fever, psittacosis, and *Coxiella burnetii* (ref. 154). Other microorganisms sensitive to BPL are *E. coli*, *Bacillus proteus*, *S. aureus*, *B. subtilis* var. *niger* spores, *Aspergillus niger*, *Microsporum audouini*, *richophyton mentagrophytes*, Coxsacki virus, poliomyelitis (Type II), and rabies virus (ref. 151). *B. subtilis* var. *niger* spores have been found to be 4 to 5 times more resistant to BPL than the vegetative cells of *Micrococcus pyogenes* var. *aureus*.

Penetrability

Beta-propiolactone exhibits poor penetrating qualities, and as such, is suitable only as a surface sterilant.

Materials Compatibility

The damage caused by BPL to various materials is attributed not to the vapor, but to the liquid form or aqueous solution. Using the recommended concentrations, exposure times, temperature, and relative humidities, no observable material damage has been observed when the vapor was used (refs. 149 and 150). It is difficult, however, to prevent the accumulation of liquid BPL condensates with the disseminators that are commercially available. The types of degradation caused by liquid BPL include: deterioration of neoprene products, slight oxidation of copper and brass, acceleration of galvanic corrosion, and dissolution of paints, lacquers, and waxes (refs. 149 and 150).

Equipment, Apparatus, and Method of Use

There are two methods used to disperse BPL. In the first case, liquid BPL is atomized by conventional aerosolizers. The required humidity is obtained by use of a steam vaporizer. In the second case, an aqueous solution of BPL is vaporized to raise the humidity and disperse the BPL in one operation. The first method is best for large enclosures, since it decreases the probability that liquid BPL will collect on surfaces and cause corrosive damage. In either case, air must be circulated to prevent oversaturation near the dispenser causing the formation of liquid droplets. Increasing the temperature of the enclosure also reduces the risk of condensation (ref. 149).

Advantages and Limitations of Beta-Propiolactone Decontamination

The advantages of using BPL for decontamination and disinfection are that: BPL is a very strong antimicrobial agent; the gas acts very rapidly at optimum RH; with proper application, residuals can be prevented; and BPL is more effective at lower temperatures than the other sterilizing gases and vapors.

Use of BPL is limited by the lack of vaporizers that will provide small enough droplets. The large droplets settle out, accumulating the liquid (which can act as a solvent on certain materials) on surfaces. In the same vein, very pure BPL (with concentrations above 97 percent) should be used to avoid the formation of oily residues from impurities. Another disadvantage is the toxicity of BPL, which is of two types. The liquid is a strong blistering agent and direct contact must be avoided. Also, dilute solutions of BPL, used for subcutaneous or topical applications, have produced tumors in rats and mice (ref. 149). This has led to severe restrictions on its use under the Federal Pesticide Act. For the most part, properly trained people can administer the vapor so that the formation of the harmful liquid condensate is minimal.

Applications of Beta-Propiolactone

Beta-propiolactone has been used to decontaminate a variety of enclosed spaces. These spaces include operating rooms and patient wards (ref. 155), a 50,000 cu. ft. Army barracks (ref. 148), a hospital dayroom (ref. 153), an 89,000 cu. ft. hospital ward

(ref. 151), maternity wards, and a vaccine production facility (ref. 130). The treatment was successful in all cases. As previously mentioned, aqueous solutions of BPL have also been used to treat blood or blood plasma in attempts to reduce the occurrence of infections resulting from transfusions.

OTHER GASES

Methyl Bromide

Methyl bromide (CH_3Br) boils at 46°C , is slightly soluble in water, and freezes at -93°C . Like other alkylating agents, it kills microbes, but it is only one-tenth as active as ETO. It is most commonly used as a gaseous contaminant to destroy organisms such as fungi, yeasts, coliforms, salmonellae, anthrax spores, and intestinal pathogens (ref. 130). Like ETO, it is slow acting. It is usually employed in concentrations of 3500 mg/l of air (ref. 153) and has the same humidity requirement (30 to 50 percent) as ETO. It is the best penetrant of all the gaseous sterilants. It is essentially a nonflammable gas and can be advantageously mixed with ETO. This mixture (60 percent MeBr and 40 percent ETO) is also nonflammable and is more penetrating and as lethally effective as ETO. Methyl bromide by itself is used to sterilize imported wool and hair that frequently contain anthrax spores (ref. 156). It has also been effectively used in the disinfection of ship compartments (ref. 139).

Propylene Oxide

Propylene oxide ($\text{C}_3\text{H}_6\text{O}$) possesses properties similar to ETO, but is less volatile and less biologically active (refs. 157 and 158). Propylene oxide (PPO) boils at 33.9°C , is 40.5 percent soluble in water at 20°C , and freezes at -112°C . It is combustible in air at concentrations of 2.1 to 21.5 percent by volume, which may or may not require dilution with inert gases for safe use (ref. 130). It forms propylene chlorohydrin that is toxic in the presence of salt or chlorine containing plastics.

The lethal efficiency of PPO is about one-fourth that of ETO (ref. 130). As with MeBr, this limits its use as a sterilant; however, it is able to penetrate most porous materials. There is some conflict in the literature regarding the relative humidity requirement, but it is believed that a range of 25 to 60 percent is adequate if treating nondehydrated materials. The

equipment needed is similar to that required for ETO. PPO is used for the decontamination of spices, powders, and flaked foods (ref. 130). Applications of PPO in the food industry have increased since the 1958 Food Additive Amendment to the Food, Drug, and Cosmetic Act ruled out the use of ETO for sterilizing food (refs. 139 and 158).

Ozone

Ozone (O_3) boiled at $-111.9^{\circ}C$, is only slightly soluble in water, and supports combustion better than oxygen. It is generated by a continuous, high-voltage discharge through air between metal electrodes. Air treated in this way can contain up to 1 percent ozone (ref. 138). In the generation process, oxides of nitrogen are formed and supplement the toxicity of ozone itself. Ozone decomposes to oxygen in the presence of moisture and is twice as stable at a relative humidity of 50 percent than at an RH of 89 percent.

Ozone causes death by the destruction of dehydrogenase enzymes. This action disrupts cellular growth. As such, microorganisms are susceptible to inactivation in varying degrees (ref. 138). *B. subtilis*, *Bacillus mesentericus*, and *S. aureus* are most susceptible; and *Achromobacter* and *Pseudomonas* generally are most resistant (ref. 159). Fungi, for the most part, are more resistant than bacteria. Organic matter can afford protection and this limits the use of ozone as a surface decontaminant.

Concentrations used for ozone decontamination have ranged from 0.001 mg/l to 5 mg/l. It is difficult to define an optimum concentration since activity is dependent of the type of microorganisms, relative humidity, level of organic protection, and stage of growth. Temperature also modifies the biological activity, with $0^{\circ}C$ being more effective than $20^{\circ}C$ (ref. 159). The optimum relative humidity range is 60 to 80 percent, which is surprising in view of the accelerated rate of ozone decomposition at higher humidities (ref. 160). The time interval for effective decontamination has ranged from 5 minutes up to an hour.

Ozone is a strong oxidizing agent. It can damage rubber and is toxic to humans. It has been used to disinfect swimming pools, since it leaves no toxic residues as chlorine does; however, it is not long lasting (ref. 138). It is used in food preservation to prevent the surface growth of mold on the outside

of eggs (ref. 159), but it is not as effective with meat. Decontamination of polarized glasses used to watch 3-D movies has been reported (ref. 161). A synergistic effect was reported when ozone, after a brief exposure to ETO, was used to sterilize an artificial heart-lung machine (ref. 162).

Peracetic Acid

Peracetic acid (CH_3COOOH) is soluble in water, melts at $0.1^{\circ}C$, and boils at $105^{\circ}C$. As a vapor, peracetic acid (PAA) is effective against most microorganisms. Vapors are generated from a commercial peracetic compound, which is 40 percent PAA, 5 percent hydrogen peroxide, 39 percent acetic acid, 1 percent sulfuric acid, and 15 percent water. It is active at an exposure concentration of 1 mg PAA per liter of air. Relative humidity at 80 percent is optimal. Because PAA is extremely fast acting, surface decontamination can be accomplished in 10 minutes (ref. 163). However, its use as a surface decontaminant is limited because it is extremely corrosive. It is a very poor penetrant.

PAA has the advantage of nontoxic decontamination byproducts (acetic acid, water, and oxygen); therefore, it has been used in the preservation of tomatoes (ref. 164), eggs (ref. 165), and strawberries (ref. 166). It is also used in gnotobiotic (germ free) research, where corrosion is avoided by the selection of resistant materials for animal cages and other equipment (refs. 167 and 168).

GAS MIXTURES

Synergism is defined as a reaction by two or more substances acting together, whose effect is greater than that produced by either of them acting alone. ETO and MeBr mixed together have been reported to be synergistic (ref. 169). Russian scientists report that the mixture of 40 percent ETO and 60 percent MeBr acts five times more rapidly on *S. aureus* and 2.5 times more rapidly on *Bacillus* spores than a mixture of 12 percent ETO and 88 percent Freon-12. The mixture is nonexplosive, even though the ETO content is fourfold higher than normal.

Studies in the United States (ref. 170) have confirmed that the ETO-MeBr mixture is faster acting than a mixture of 12 percent ETO with 88 percent Freon-12 in the sterilization of plastic materials.

The synergistic effect is probably not based on an increased rate of ETO sterilization, but on an increased rate of penetration, at least in the plastics studied (ref. 171). Without plastic barriers, the effect is not synergistic, but additive.

Formic acid (HCOOH), as a vapor or as a solution, has also exhibited a synergistic effect with ETO (ref. 172). Tests using *B. subtilis* spores have indicated that the maximum effect is observed with formic acid vapor at a concentration equal to one-tenth its saturated vapor pressure. Higher concentrations do not increase the effect. The synergistic effect allows either the use of less ETO (up to two-thirds the usual dosage) or a decrease in the exposure time. The results also indicated that formic acid, at the concen-

trations and exposure times used in the study, did not adversely affect plastics and metals.

Ozone and ETO have been used in combination, although not simultaneously (ref. 139). It was found that a brief exposure to ETO followed by ozone treatment resulted in a synergistic effect.

In the future, the use of gas combinations for sterilization will probably be more frequent. More research is required on the effects of temperature and relative humidity and on the optimum concentration of each gas component before the maximum synergism can be achieved. If these efforts are successful, products will be able to be sterilized under conditions that significantly reduce the stress now encountered in normal gas sterilization cycles.

PART 2

RECENT ALLIED TECHNOLOGICAL DEVELOPMENTS

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CHAPTER 6

Sterilizable Materials, Parts and Components

"They trimmed its frisky whiskers with a pair of hard-boiled shears"

INTRODUCTION

For many years, various forms of heat have been used to sterilize purely mechanical parts, such as surgical instruments, laboratory glassware, and the hard-boiled shears referred to above. Until the NASA Viking Program, the incredibly complex maze of electronic and mechanical devices comprising a typical spacecraft could not be effectively sterilized. Their performance was impaired by the application of traditional heat-sterilization techniques that were too harsh for the materials to withstand.

Along with the new sterilization techniques developed during the Viking Program and discussed in previous chapters, NASA had to design and produce complex equipment that was compatible with these techniques and that could also withstand the space environment.

Such necessary items as tape recorders and stereophonic equipment consist of a multitude of electronic parts such as resistors, capacitors, and transistors. An ordinary tape recorder in the home usually confronts no extremes of temperature or humidity; however, a similar tape recorder aboard the Viking that landed on a biologically interesting planet faced other conditions and had to be sterilized. NASA had to develop a sterilizable tape recorder and hundreds of other complex devices before it could assemble a totally sterile and functional spacecraft.

In 1963, the Jet Propulsion Laboratory initiated a program to define and solve the problems associated with the heat sterilization of a typical spacecraft magnetic tape recorder. All the recorder components and materials were carefully selected for their ability to tolerate the sterilization environment, and a comprehensive test program was conducted to verify their sterilizability. Based on the test results, a complete tape recorder was assembled from parts that, when exposed individually, had all demonstrated their ability to withstand the sterilization

environment. The completely assembled tape recorder was sterilized in a dry-heat environment and then tested. After only 10 days, the recorder bearings failed as a result of chemical interactions during sterilization. This indicated that the availability of sterilizable parts afforded no guarantees for a total sterilizable system. NASA was indeed faced with a complex problem.

NASA ultimately solved its tape recorder problem. In fact, extensive developmental test programs were conducted to determine and improve the sterilizability of materials, parts, components, and systems. Comprehensive information detailing the effects of sterilization on the physical and functional properties of parts and materials is now available. By proper selection of parts and materials and appropriate design consideration, products now can be designed to withstand sterilization and provide greater service and reliability at elevated temperatures.

ETHYLENE OXIDE STERILIZATION

Early in the NASA Viking Program consideration was given to the use of gaseous sterilization that was being widely used in the pharmaceutical industry. After surveying the various possible gases (as discussed in chapter 5), NASA decided that ETO held the most promise for aerospace application and a fairly extensive test program of parts and materials compatibility was initiated.

Effects of ETO on Parts and Materials

ETO is capable of modifying the physical and chemical properties of many compounds. It can change material properties by reacting directly with base materials or with impurities resulting from the manufacturing process. In the vapor phase ETO is highly penetrative. Freon-12 (used in conjunction with ETO) has a tendency to produce swelling in some elastomers.

Liquid ETO is an excellent organic solvent and, therefore, it can damage materials. It acts as a solvent on such acrylic plastics as Lucite⁽¹⁾ and Plexiglas⁽²⁾ and will attack plasticizers used for binding. It will also swell and blister rubber.

Testing of materials, components, and systems for compatibility with ETO sterilization involves not only surface effects, but also deep penetration effects on solid, porous materials. Solid substances such as plastics, rubber, and leather will absorb ETO during exposure. Retention of the ETO gas may increase its corrosive effects on certain components; however, elevated temperatures contribute to the removal of absorbed ETO, and tend to minimize the ETO effects.

Materials that must not be used with ETO include copper and copper alloys, silver and silver alloys, magnesium and magnesium alloys, and steel or iron containing any trace of rust. Both silver and copper can catalyze polymerization of ETO if moisture is present. Rust, aluminum salts, and caustic salts catalyze rapid polymerization of ETO. This often leads to rapid heating, which will cause violent reactions.

The compatibility of ETO-Freon mixtures with polymers is of some concern. Either by chemical reactions or by absorption, ETO can react with polymers to affect the material properties. Although some base polymers may be stable in the presence of ETO, ingredients such as pigments and fillers, added to the polymer to alter its characteristics, can also affect its reaction with ETO. Any chemical reaction between ETO and a polymer, or any of a polymer's modifying ingredients, is accelerated by heat.

The hazards of ETO exposure are: corrosion and electrical effects (the breakdown of insulation characteristics) due to humidity or condensed water combined with elevated temperatures, precipitation of ETO polymers (which are corrosive) caused by the catalytic properties of certain materials, and the possibility of chemical reactions between the constituents of the chamber environment and the materials exposed (leading to eventual corrosion of the chamber and degradation of the materials) (ref. 173).

(1) Trademark of I.E. DuPont de Nemours Co.

(2) Trademark of Rohm and Haas Co.

ETO AND DRY-HEAT STERILIZATION

Because the high humidity required to obtain adequate kill with ETO corroded certain internal spacecraft materials, NASA considered opting for the dry-heat process in its place. However, prior to abandoning the gaseous sterilization concept, NASA investigated the combination of ETO and heat in an attempt to reduce the severity of each process, while maintaining the assurance that proper sterilization was achieved.

Again, a parts- and materials-compatibility test program was performed. Although NASA finally elected to use dry heat alone, significant knowledge was obtained. This knowledge is applicable to many commercial operations that do not require the high, long-term reliability needed for spacecraft devices and for which the sensitivity of parts is not critical.

Effects of ETO and Dry Heat on Electronic Components

Electronic modules of proven design have been tested to determine their ability to withstand ETO/dry-heat sterilization (ref. 174). The modules were exposed to separate ETO and heat-sterilization cycles. The ETO environment did not produce any apparent ill effects on the modules. Some decrease in insulation resistance occurred immediately after exposure to ETO because of the moisture absorbed. After exposure to ambient conditions for 24 hours or more, resistance returned to near normal; therefore, electronic devices should be allowed to stabilize after exposure to ETO.

Heat sterilization caused discoloration of the polyurethane conformal coating and filleting materials. The change in color was due to additional curing (further polymerization). There was no evidence of harm to the electrical properties.

A small percentage of the components tested exhibited electrical failures. One failure was attributed to packaging design. For example, several transistor leads were lifted from their pads. This type of failure can be eliminated by proper design and selection of encapsulants.

Of the modules tested, capacitors were the only electronic components that failed because of the heat environment. Both wet tantalum and polystyrene

dielectric capacitors failed. These failures are not surprising, since both types of capacitors are inherently sensitive to heat.

Tests of high-reliability electronic assemblies revealed them to be capable of withstanding the combined effects of ETO and heat sterilization. No general conclusions should be drawn, since only a limited number of units were tested, and since these consisted of components designed to withstand severe environments.

Effects of ETO and Dry Heat on Polymers and Metals

Tests (ref. 175) were performed to study the effects of ETO and heat on polymers and metallic surfaces. Sixty-eight commercial polymeric products were exposed to heat and ETO sterilization. Physical, mechanical, and electrical tests were performed on materials to determine post-sterilization degradation and to establish product compatibilities.

Although all products showed a change in properties after gas exposure and an additional change after dry heat, only two were rated "not compatible" and 18 were rated "marginal." None showed an appreciable loss of electrical properties.

Other material-compatibility tests (ref. 176) included plastics, potting materials, elastomers, and metal coatings. No change in appearance was observed, and physical, mechanical, and electrical properties were only slightly affected.

Tests were also conducted on resistors, capacitors, R. F. coils, connectors, motors, and magnetic tape. The resistors, capacitors, and R. F. coils showed slight property change. Stepper motors displayed no significant difference in stall current, stall torque, dielectric standing voltage, or insulation resistance.

Although the list of materials and test results is too extensive to include in this document, the amount of data accumulated does point out that a large selection of materials, parts, and components suitable for sterilization are available.

Effects of ETO and Dry Heat on Material Combinations

Studies (ref. 177) were conducted to examine typical, sterilizable spacecraft material combinations that were considered potentially incompatible. Each material was combined with a second material and, while in intimate contact, was subjected to ETO and

heat sterilization. Table 10 lists the materials tested. Physical, mechanical, and electrical property tests were performed on each product before and after sterilization to measure material stability. The adhesive property of the polyurethane or the silicone polymeric materials was not affected.

The combinations of Mylar with Solithane and Mylar with DC 93-500 were incompatible based on the loss in tensile strength and ultimate elongation that occurred in the Mylar. It was noted that the Solithane strongly adhered to the surface of the Mylar. The Mylar-Micarta H5834 combination was found to be compatible. The loss of Mylar tensile strength and elongation was comparatively slight. Previous studies have shown Mylar to be only slightly affected when exposed alone. The apparent conclusion is that Mylar underwent additional degradation when sterilized while in contact with a second material.

The only structural combination that was found to be incompatible was thermoplastic polyphenylene oxide. The polyphenylene oxide showed a significant loss in strength and became brittle.

Overall the material-compatibility tests showed 17 percent of the materials to be incompatible. This may appear to be significant, but it must be remembered that the material combinations were selected as "worst case" combinations. Therefore, it was concluded that material incompatibility in material combinations should not be an overriding concern in using ETO/dry-heat sterilization.

DRY-HEAT STERILIZATION

The NASA Viking Program represents the largest program ever undertaken to sterilize complex aerospace equipment. The goal of Viking was to land a completely sterile package to conduct sophisticated scientific experiments on the planet Mars. This equipment had to not only survive the rigors of space flight, but also withstand the sterilization process here on Earth.

Viking Sterilization Philosophy

At the outset of the Viking program, a basic decision was made to accept dry heat as a terminal sterilization process. Based on prior research and experience, a dry-heat environment (nitrogen) of 125°C for 60 hours was selected to qualify

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TABLE 10. - *Test Material Matrix*
(From ref. 177)

Test Material	Secondary Material	Polymer Type	Use
Epon 828/Z	Solithane 113/300	Epoxy/Polyurethane	Adhesive/Coating
Epon 828Z	DC93-500	Epoxy/Silicone	Adhesive/Encapsulant
Mylar 100A	Solithane 113/300	Polyester/Polyurethane	Film/Coating
Mylar 100A	DC93-500	Polyester/Silicone	Film/Encapsulant
Mylar 100A	Micarta H5834	Polyester/Phenolic	Film/Hardware and Structural Parts
Kapton 200X H667	Micarta H5834	Polymide/Phenolic	Film/Hardware and Structural Parts
Kapton 100X H667	Solithane 113/300	Polymide/Polyurethane	Film/Coating
Kapton 200X H667	DC93-500	Polymide/Silicone	Film/Encapsulant
PPO 534-801	DC93-500	Polyphenylene Oxide/Silicone	Hardware & Structural Parts Encapsulant
Micarta H5834	Solithane 113/300	Phenolic/Polyurethane	Hardware & Structural Parts/Coating
Micarta 20201-2	Solithane 113/300	Silicone/Polyurethane	Hardware & Structural Parts/Coating
Micarta H5834	DC93-500	Phenolic/Silicone	Hardware & Structural Parts/Encapsulant

components. Parts and materials were subjected to a more stringent qualification criterion of 135°C for 100 hours, to assure an adequate margin of safety.

The basic philosophy used in the design of the Viking was to subject candidate materials and parts to a more extreme sterilization cycle than that used in the flight acceptance testing (FAT) of flight hardware, or terminal sterilization of the entire Viking Lander Capsule (VLC). It was hoped that with this procedure basic materials and components that were marginal or unacceptable in the sterilization environment could be eliminated early in the program with a minimum of retrofitting or redesign.

From the extensive sterilization qualification testing (refs. 178 and 179), a list of basic materials suitable for design parts, components, and subsystems evolved. Excluding metal alloys, 813 candidate materials were tested and 420 were found to be acceptable for use in the dry nitrogen environment at 135°C for 100 hours.

Nonmetals

To assure that materials used in the hardware phase of Viking were identical to initial lot/batches of qualified materials, nonmetallic materials bought later were subjected to a chemical "fingerprint" test upon arrival. In this manner, all new lots of material were compared with the original qualification lot to ascertain that the material and its processing were chemically identical to the original qualification lot. Because of the 10°C safety margin over component tests, and the 23°C safety margin over system testing, the materials program proved to be highly successful in providing materials for the successful design of sterilizable components such as tape recorders, computer subsystems, batteries, landing radar, and a variety of chemical, biological, and physical measurement instruments.

A listing of the types of materials evaluated for use on Viking is given in table 11. This listing includes both successful and unsuccessful candidates.

Because so many nonmetallic products were evaluated for their compatibility with the heat-sterilization requirements of Viking, it would be difficult to draw any generalizations about the suitability of any one specific class or type of compound. Accordingly, a list of the more common

types of nonmetallic materials, along with the measured onset of thermal degradation, is cited in table 12 (page 107). This table of previously unpublished data* is included merely to show the large number of plastics, adhesives, and rubbers that are compatible with heat-sterilization environment.

Caution is advised in using the table because the temperatures cited for onset of thermal degradation were determined by measurement of weight loss. For many materials, changes in properties may occur due to elevated temperature exposure, which do not result in material weight loss. Conversely, many materials may undergo a weight loss at elevated temperatures, such as the splitting of water in the cross-linking of phenolic polymers, which produces a stronger, more highly cross-linked product. Finally, some materials undergo a weight loss of extraneous material, such as unreacted monomer and catalyst, which produces no measurable change in other properties of the material. This list is not intended as a recommendation or condemnation of any specific material, since under different processing and curing conditions, many of the materials would indicate either better or worse thermal stability.

Metals

Many aluminum alloys are compatible with the heat sterilization environment. The 2000 series aluminum alloys were used extensively throughout the Viking Lander because of a minimal effect on their room-temperature properties. The frequently used aluminum alloy, 7075, was found to overage during the sterilization sequence, causing a 10 percent reduction in properties.

Beryllium should pose no significant problem; however, its low-thermal expansion creates high stress when assembled to larger aluminum or magnesium parts.

Magnesium alloys have limited use in sterilizable hardware because of their high creep rates under low stress and their loss of room-temperature strength after exposure to elevated temperatures. An attempt

*These data were compiled by Stan Fudlaseck in 1976 at the Materials Research Laboratory of Martin Marietta Corporation, Littleton, Colorado.

TABLE 11. — *Quantity of Nonmetallic Materials Chemically Tested (From ref. 178)*

Generic	Quantity	Application	Quantity
Acrylics	2	Ablator	13
Butyl Rubber	6	Adhesive	127
Diallyl Phthalates	18	Coating	73
Epoxies	307	Elastomer	64
Ethylene Propylene Rubber	5	Electrical Insulation	110
Fluorocarbons	60	Encapsulant	65
Miscellaneous (principally inorganic nonmetallics)	92	Fabric	57
		Film	25
Phenolics	13	Finish	12
Polyamides	20	Lubricant	24
Polycarbonates	1	Marking	26
Polychloro-P-Xylene	2	Miscellaneous	20
Polyester	43	Molding Compound	9
Polyethylene	5	Sealant	36
Polyimide	45	Shock Insulation	10
Polyolefin	2	Structural Plastic	45
Polypropylene	7	Tape	18
Polystyrene	5	Thermal Insulation	48
Polyvinyl Chloride	1	Tubing	13
Polyvinyl Fluoride	6	Wire	18
Silicone	123		
Unknown	11		
Urethane	39		
Total	813		813

was made to use A231 B magnesium alloy for packaging the data acquisition processing unit on Viking. Because of excessive creep during sterilization, the box fasteners failed. In a modified design, magnesium-thorium HM-21 and HK-31 alloys performed satisfactorily.

Titanium alloys are well suited to spacecraft applications because they can withstand heat sterilization with little effect on their room-temperature properties. If these alloys are used in combination with magnesium or aluminum, their low-thermal expansion induces mechanical interface loads. During sterilization, extended heating and cooling times can result from the low-thermal conductivity of titanium alloys.

Heat sterilization has little effect on the room-temperature properties of most steel alloys, unless temperatures are elevated; then the structural properties are reduced and should be considered in the design of preloaded structural members.

Effects of Dry Heat on Mechanical Parts

Bearings.—Bearings and lubricants that can withstand thermal sterilization are available. The use of high-temperature lubricants is rather common.

Springs.—During thermal sterilization, springs undergo their maximum stress because of both the thermal loads and the normal spring loads. The use of heat sterilization is not recommended for common spring material i.e., hard-drawn steel and music wire.

Soldered Connections.—Sterilization can seriously affect the reliability of electrical connections, therefore, attention must be given to the metallurgical effects on the alloys used in the solders. For example, some soldering compounds, when exposed to high temperatures for extended periods of time, undergo property changes and sometimes lose their strength.

One area of particular concern was whether eutectic solder could withstand high sterilization temperatures. High-temperature solders currently exist; nevertheless, the application of such solders at high temperature poses additional problems of fabrication and assembly.

Soldered connections are the most vulnerable to heat. The commonly used tin-lead solders have a solidus temperature of 180°C and liquidus temperature of 205°C to 240°C. Different formulations affect the temperature properties and tensile strength of the joint. Addition of antimony in the alloy increases the strength of the alloy by almost 30 percent. Tests indicate that no significant changes in physical or electrical properties occur in soldered joints after thermal processing. The rupture strength of soldered joints may be reduced as much as 25 to 30 percent during high-temperature sterilization, but no rupture of the joint normally occurs if the stress on the junction is not excessive.

Using the appropriate stress relief bends and filled solder holes, soldering techniques used on Viking (ref. 180) were found to have unlimited life at 150°C.

Some other mechanical parts that have demonstrated the ability to withstand heat sterilization are connectors, cabling, switches, transducers, and electronic packaging hardware.

Printed Circuit Boards.—Some problems were experienced in the Viking program with multilayer printed circuit boards. It was found that inadequately cured resins were causing openings in the plated holes during sterilization. When properly processed, printed circuit boards, both single- and multi-layer, were found to be entirely compatible with the heat sterilization cycle.

Effects of Dry Heat on Electronic Parts

Extensive tests were conducted for Viking and in other programs (ref. 181) on the performance and reliability of electronic parts after dry-heat sterilization. The parts considered were capacitors, resistors, variable trimming resistors, transistors, transformers, and diodes. The primary purpose of the tests was to establish a list of heat-sterilizable electronic parts, that could be used in spacecraft sterilization. The parts listed were required to meet stringent criteria and were subjected to rigid controls and tests. General reliability data on 72,846 parts made up of 577 part types were accumulated.

Capacitors.—Of the many types of capacitors tested for Viking, ceramic capacitors experienced the least number of catastrophic failures. In order of best performance, the various dielectric types of capacitors were ranked as follows: (1) ceramic, (2) glass, (3) solid tantalum and foil tantalum, (4) plastic, and (5) Mylar.

Resistors.—The tests revealed that resistor failure is mainly related to type of resistor (i.e., wirewound, metal film, etc.) rather than to resistance value or wattage rating. Based on Viking test results the ranking of the resistors for overall performance was: (1) carbon film, (2) metal film, (3) precision power wirewound, (4) precision wirewound, and (5) carbon composition.

Variable Trimming Resistors.—Variable trimming resistors were found to be extremely stable in total resistance and voltage ratio. Significant variances in stability occurred. The carbon variable trimming resistors were the least stable of all and exhibited the largest amount of total resistance drop. Most of the failures observed were attributed to one man-

ufacturer; consequently, the manufacturer's design and process control is a major factor in the selection of this type of resistor.

In order of best performance and lowest failure rate, the ranking of variable trimming resistors tested was: (1) wirewounds, (2) carbon, and (3) metal film.

Diodes.—Many types of silicon diodes were tested for use on Viking. The devices tested included: high-voltage, high-current diodes; low-voltage, high-current diodes; and high-voltage, low-current diodes. Because of the significant failures occurring in the high-voltage, high-current devices, these units are not being used for spacecraft application.

In order of best performance and lowest failure rate, the ranking of diodes tested was: (1) high-voltage, low-current diodes; (2) low-voltage, high-current diodes; and (3) high-voltage, high-current diodes.

Digital Monolithic Microcircuits.—Seven digital monolithic microcircuit part types, including three types of gates and four types of flip-flops, were tested. The preliminary test results indicated that no significant failures resulted from heat sterilization. A definite reliability problem was observed with the microcircuits, but process controls during manufacturing were found to be of more concern than the hazard of sterilization. Screening tests are strongly recommended.

Transistors.—Most failures noted during transistor tests were results of mechanical production line and contamination problems. No significant differences between the sterilized and nonsterilized units were noted, which indicated that sterilization environments pose no major problem to transistors. Of the types tested, the NPN signal and medium-power transistors appeared to be the most reliable.

Transformers and Inductors.—Sterilization caused considerable damage to some of the transformers and inductors tested. Extensive cracking of the rigid epoxy occurred from the stress of thermal expansion. The cracking occurred mainly on the larger units that used a large volume of material. Smaller, potted units of the same material did not crack. Considerable electrical-parameter deviations were also caused in all of the transformers and inductors. The deviations were not considered too significant, since initial system design can allow for them.

In summary, the testing has resulted in an extensive list of highly reliable piece parts, capable of withstanding sterilization environments. The parts expe-

riencing the most deleterious effects were capacitors and transformers.

Effects of Dry Heat on Components and Devices

Further testing during the Viking program resulted in the development of complex, heat-sterilizable components and subassemblies. These components include tape recorders, telecommunication equipment, airborne computers, gyros, accelerometers, data acquisition units, parachute material, and propulsion systems. Devices include valves, motors, relays, actuators, and pyrotechnics. The development of these articles demonstrates that almost any type of equipment, including highly sophisticated electronics and intricate mechanical assemblies used in liquid propulsion systems, can be designed to withstand heat sterilization. Photographs of some of the items discussed are shown in figures 35 through 41.

APPLICATIONS

Comprehensive information detailing the effects of sterilization on the physical and functional properties of spacecraft parts and materials is now available.

The development of sterilizable parts, materials, and components has many direct applications. Improved surgical materials and equipment, sterilizable monitoring equipment for operating rooms and burn wards, and other medical applications are evident. Sterilizable implantables, such as heart

pumps with higher reliability and improved miniaturization, are also being perfected.

Perhaps the greatest benefits will result from indirect application. For example, improved plastics and bonding agents have been developed to withstand elevated temperatures. These compounds exhibit easy application, good adhesion, flexibility over wide temperature ranges, nonflammability, and good electrical properties. Potential uses include fire-retardant coatings on electronic devices and other products requiring heat-protective coatings.

The commercial applications of this NASA-developed knowledge are far reaching. Cookware that can better withstand oven temperatures and a multitude of home appliances (such as motors, insulating material, and electrical parts) that are degraded by heat can be improved and made more reliable. One of the major causes of failure in automobile parts is the effect of heat. Repair costs can be minimized by production of such items as heat-resistant hoses, gaskets, seals, electrical wiring, heater parts, and radiator parts. Major engine and transmission repairs may be reduced, thereby extending the life of the parts. Improved brake systems that result in greater safety and reliability and the safety of commercial aircraft equipment may also result from these developments. Additional industrial applications include improved products such as relays, motors, transformers, activators, controls, and monitoring equipment that must operate at elevated temperatures.

TABLE 12. — *Dry-Heat Compatibility of Nonmetallic Materials*
(Source—Materials Research Lab, Martin Marietta Corp.)

Material	Manufacturer	Onset of Thermal Degradation, Temperature, °C
1. Epoxylite 6107	Epoxylite Corp.	165
2. Eccosorb MF-124	Emerson & Cuming	330
3. Epon 901/B-3	Shell	295
4. Ablefilm 517	Ablestik	225
5. Scotchweld 583	3M Co.	230
6. Glass Fiber Insulation Type PF 105-700	Hitco	135
7. Stycast 1095	Emerson & Cuming	210
8. Armstrong A-2/ Activator E	Armstrong Products	215
9. FM96U	American Cyanamid	135
10. HT435	American Cyanamid	170
11. STM-E-721-22-4 Wire Insulation	Haveg	470
12. STM-E-718-22-9 Wire Insulation	Milo Carolina	430
13. G125800 Bioshield	Schjeldahl	315
14. G125900 Bioshield	Schjeldahl	325
15. GE-TB5-758-A-MMC Thermal Coating	MMC	135
16. F411 Lacing Cord	Western Filament	390
17. Min-K-1301	Johns-Manville	350
18. E-515-8, Ep Rubber	Parker Seal	260
19. Penntube WTF	Penntube Plastics	425
20. Markem Ink 6832G 1510 Blue Ty I	Markem Corp.	55
21. Markem Ink 6832G Blue Ty IV	Markem Corp.	55
22. Dixon Pencil Blue Number 783	Dixon	135
23. Adlock	American Reinforced Plastics	325
24. Penntube I	Penntube Plastics	485
25. SLA-561V	MMC	205
26. Eccosorb MF500F-116	Emerson & Cuming	190
27. HX1000 Fluorocarbon Bonding Film	3M Co.	315
28. Dacroncloth, Vydx 550 Coated	Goodyear	235
29. AMS-3302 Silicone Rubber	Parker Seal	370
30. Gold Coated Kapton	National Metallizing	415
31. SLA-220V VAP 2/1	MMC	330

TABLE 12. — *Dry-Heat Compatibility of Nonmetallic Materials* (Continued)

Material	Manufacturer	Onset of Thermal Degradation, Temperature, °C
32. Epoxy Enamel, White	Andrew Brown	305
33. Epoxy Enamel, Black	Andrew Brown	315
34. Choseal 1236	Chomerics	385
35. E787 Epoxy Prepreg	U. S. Polymeric	195
36. Kapton F919	DuPont	410
37. Kapton F011	DuPont	420
38. Aluminized Polyester Film	King-Seeley	180
39. Dacron Web GER 15020	Goodyear	235
40. BTR Silicone Rubber	Lord Mfg.	135
41. Kapton, Gold Coated	Standard Packaging	425
42. Kapton, Gold Coated	Schjeldahl	450
43. Consil Gasket	Tecknit	135
44. Insultek 345-D, Black	IERC	180
45. Stycast 36D	Emerson & Cuming	135
46. Eccosorb CR-124	Emerson & Cuming	325
47. CR MM-350 High Modulus Teflon	Chemical Hard Rubber	500
48. Dickson Marking Ink, Black	Specialty Ink Co.	50
49. PC 12-007	Hysol	260
50. Fluoroglass E650	Dodge Industries	500
51. HT424 Primer	American Cyanamid	170
52. Wire STM-E-719-1A-16-9	Tensolite	400
53. Tufram H-2 Grade 3	General Magnaplate	275
54. DC92-007, Gray	Dow Corning	150
55. RTV 511, Gray	GE	275
56. RTV 511, Black	GE	80
57. Min-K, Type SS	Johns Manville	700
58. TFE, Etched	Goodyear	480
59. Wire, STM-E-922, 20-0, Rev 2	Haveg	420
60. Polythermalized Magnet Wire	Belden	355
61. Abletherm 7-1	Ablestik	175
62. Thermamid Insulated Wire	REA	335
63. EA 9414	Hysol	150
64. Vydex 550	DuPont	110
65. Kynar Tubing	Raychem	260
66. Nomex Cloth GER15311	Goodyear	285
67. Dacron 61D A16/070	GAF Corp.	135
68. GEE FR-4 MIL-D-18177C	Electroly Inc.	280
69. FR-4 FL-GF	Electroly Inc.	275
70. GEE FR-4 Unclad	Sythane-Taylor	300

TABLE 12. — *Dry-Heat Compatibility of Nonmetallic Materials (Continued)*

Material	Manufacturer	Onset of Thermal Degradation, Temperature, °C
71. GEE FR-4 Unclad	Nelco	285
72. FR-4 FL-GR Clad	Nelco	135
73. GEE FR4 Unclad	The Mica Corp.	260
74. Translube	Cherry Rivet	320
75. Insultek 445	IERC	155
76. ES218 Epoxy	Poly Scientific	315
77. Dacron Cloth/Markem Ink	1088/1059	115
78. FR-4 FL-GF Clad	The Mica Corp.	135
79. FR-4 FL-GF Clad	Synthane-Taylor	285
80. Kynar Sleeve—No Melt Liner	Raychem	365
81. Kynar Sleeve—With Melt Liner	Raychem	375
82. S-469-4 Silicone	Parker Seal	400
83. Nomex Cloth/Vydar	Goodyear	390
84. Glass Tape, B Weave	Essex International	180
85. Epon 828/Cat. 2/Epon 871	Shell Chemical	135
86. C100-21 Insulation Paper	Peter S. Schweitzer	135
87. Cat-L-Ink 50-100, Cat. 20	Wornow	210
88. Cat-L-Ink 50-771, Cat. 28	Wornow	210
89. Polyolefin Shrink Tube	Rayclad Tubing	135
90. Lefkoweld 46/LM52	Leffingwell	150
91. Tape Number 56	3M Co.	210
92. Tape Number 27	3M Co.	135
93. Lacing Tape, Airtex 217, Class 3	Eon Corp.	210
94. C-15-015	Hysol	135
95. Cat-A-Lac 463-3-8	Finch Paint & Chem	130
96. Aerocati Coat—Primer	Sherwin-Williams	210
97. Lacing Tape, PTFE	Dodge Industries	200
98. Number 3021 Epoxy	Epoxy Products	60
99. SU92-RER/DC307	MMC	225
100. Stycast 2651/Cat. 9	Emerson & Cuming	135
101. Stycast 2651	Emerson & Cuming	135
102. Tape Number 74	3M Co.	200
103. FEP Film	DuPont	400
104. Zyglo ZL-4B Penetrant	Magnaflux	65
105. Zyglo ZP-5 Developer	Magnaflux	250
106. Hexcel F161/181	Hexcel	300
107. FEP Type A Bonding Film	DuPont	440
108. VC-3	Nylock-Detroit	135
109. 997 Silicone Varnish	Dow Corning	135
110. Penntube SMT	Penntube Plastics	410

TABLE 12. - *Dry-Heat Compatibility of Nonmetallic Materials (Continued)*

Material	Manufacturer	Onset of Thermal Degradation, Temperature, °C
111. BenHar Pyrosleeve ST	Bentleye-Harris	810
112. Chlorotrifluoroethylene	Allied Chemical	315
113. Isomica Number 4150	Hi-Shear	135
114. BTR, E058R	Lord Mfg.	225
115. Number 79 Insulation Tape	3M Co.	150
116. STM-E-843 Wire	Haveg	425
117. STM-E-921 Wire	Haveg	500
118. E601 Clad	Dodge Industries	475
119. Eccobond 56C/CAT. 11	Emerson & Cuming	330
120. Skyspar A423	Andrew Brown	140
121. Cab-O-Sil CC1024A Class B/RE2000 Cat./M5	Dolph	125
122. BC328A/B 328C	Dolph	220
123. LBB-3 Phenolic Laminate	Sythane Taylor	175
124. ADX-41	Hysol	210
125. Cat-L-Ink 50-206/Cat 9	Wornow	155
126. Cat-L-Ink 50-407/Cat 9	Wornow	160
127. Cat-L-Ink 50-300/Cat 9	Wornow	150
128. Apiezon "N"	Apiezon Products	120
129. Dacron Cord GER15178	Goodyear	150
130. Krytox 143AY	DuPont	60
131. Krytox 240AC	DuPont	430
132. Krytox 143AX	DuPont	55
133. Solithane 291/2L271	Thiokol	200
134. EG818T Epoxy/Glass	The Mica Corp.	240
135. Eccofoam FP/12-2	Emerson & Cuming	120
136. BTU560/SS4155 Primer	GE	215
137. DC 372	Dow Corning	175
138. Rulon A	Dixon Corp.	500
139. L-P-509 Epoxy/Glass, G11	Formica	245
140. PR1538	Products Research	135
141. PR420 Primer	Products Research	150
142. Gore Type CR Wire	Gore	405
143. Dacron Cloth GER15459	Stern & Stern	190
144. Hexabond HB6831	Hexcel Corp.	140
145. Dixon Marking Pencil	Dixon Corp.	150
146. EG802T	The Mica Corp.	275
147. Number 450 Epoxy-Class Tube	3M Co.	160
148. Eccobond 57C	Emerson & Cuming	225
149. Tufram Resin	DuPont	480
150. Polycell Foam	Olin Corp.	70
151. LCA-4Y, BA-5	Bacon Ind.	280

TABLE 12. — *Dry-Heat Compatibility of Nonmetallic Materials (Continued)*

Material	Manufacturer	Onset of Thermal Degradation, Temperature, °C
152. FA-13/BA-39	Bacon Ind.	150
153. LCA-4/BA-5	Bacon Ind.	300
154. SMRD 100F50	GE Space Div.	280
155. Eccostock R20	Emerson & Cuming	305
156. Trubond 210	Fenwall	225
157. Apiezon H	Apiezon Products	150
158. CD75-171	Kirkhill Rubber	315
159. Dacron Tape GER15039	Bally Ribbon	250
160. P527 Paint	Andrew Brown	145
161. DC6-9220	Dow Corning	370
162. Fiberite MXS176	Allied Chemical	135
163. CHR-R-10470	Conn Hrd Rbr	135
164. Cat-L-Ink 50-100/Cat 9	Wornow	135
165. Cat-L-Ink 50-202/Cat 9	Wornow	135
166. Cat-L-Ink 50-507/Cat 9	Wornow	135
167. Cat-L-Ink 50-700/Cat 9	Wornow	145
168. Wornow Ink M-9-N/Cat 9	Wornow	145
169. Wornow Ink M-0-N/Cat A	Wornow	135
170. Wornow Ink M-0-N/Cat A	Wornow	135
171. EA934/Antifoam A	Hysol	250
172. Torr Seal Epoxy	Varian	230
173. DC93-072	Dow Corning	135
174. QN 887	Tapecon Inc.	265
175. Rexolite Polystyrene	Not known	340
176. Markem Ink 6832, Dacron Cloth	Markem Corp.	280
177. S1669 Silicone Rubber	Goshen Rubber Co.	305
178. SE-5211 Silicone Rubber	Reeves Rubber Co.	250
179. Poxyfilm 1001	New England Laminates	270
180. G683 Thermal Filler	GE	180
181. Fiberite MX 2625	Allied Chemical	145
182. Tape, P292	Permacel	125
183. Number 17107A Rubber	Precision Rubber	280
184. DuPont 5504A	DuPont	165
185. XC8-H789/H8L-3543	Hysol	220
186. Epo-Tek H72	Epoxy Technology	250
187. CPI-2225/181-A1100 Laminate	Ferro Corp.	355
188. Ablecast 147-1	Ablestik	240
189. Ablestik 293-1	Ablestik	150
190. SMRD 100F70	GE	135
191. Nextel 401-C10, Black	3M Co.	210
192. BTR E0435	Lord Mfg.	135

TABLE 12. — *Dry-Heat Compatibility of Nonmetallic Materials* (Continued)

Material	Manufacturer	Onset of Thermal Degradation, °C
193. Cho-Bond 1030	Chomerics	370
194. Cho-Bond 1030/ Cho-Seal 1250	Chomerics	320
195. Cho-Seal 1250	Chomerics	340
196. Cho-Seal 1250/Primer	Chomerics	355
197. DC33 Grease, Light	Dow Corning	120
198. DC33 Grease, Medium	Dow Corning	50
199. DC93-076½	Dow Corning	290
200. Number 765 Polyester Tape	Kendall Polyester	125
201. F502/120	U. S. Polymeric	390
202. F502/1581	U. S. Polymeric	355
203. Loop Tape 1299	American Velcro	245
204. RTV566/SS4155 Primer	GE	340
205. RTV 567	GE	260
206. PTFE Tape, HM 350	Conn Hrd Rbr	500
207. Viton JPM-164	Quantum Inc.	220
208. Compound 970C 1562	Kirkhill Rubber	345
209. Compound E60C	L. B. Moore, Inc.	310
210. CR124	Emerson & Cuming	320
211. DC66-1103, Lube	Dow Corning	220
212. Abletherm 7-1 SS4120 Primer	Ablestik/GE	315
213. DC11 Grease	Dow Corning	135
214. RTV 615	GE	310
215. Epocast 203	Furane	315
216. Cat-A-Lac 473-3-1	Finch Paint & Chem	125
217. Eccostock	Emerson & Cuming	135
218. Solithane 271/291/TIPA	Thiokol	150
219. 8294 Conductive Epoxy	DuPont	215
220. AI-1137 Varnish	Amoco Chem	320
221. Glass Fiber Fel., Type E	Johns-Manville	760
222. FM96	American Cyanamid	170
223. Compound 950-c-2491	Kirkhill Rubber	315
224. Nomex Type 411	DuPont	285
225. SMRD Number 100F-90	GE	140
226. Ny-032 Polyamide-Glass	Nyglass Co.	240
227. Compound Number 11054	Precision Rubber	285
228. Compound 950-C-1483	Kirkhill	150
229. Compound 950-C-1569	Kirkhill	165
230. B-400 Tape	Brady Label	150
231. ES308 Epoxy	PolyScientific	180
232. ES378 Epoxy	PolyScientific	200
233. Type 366, Cork	Armstrong Cork	135

TABLE 12. — *Dry-Heat Compatibility of Nonmetallic Materials (Concluded)*

Material	Manufacturer	Onset of Thermal Degradation, Temperature, °C
234. Fireban 600 PC Brd, Clad	Synthane-Taylor	275
235. N105 PC Brd, Clad	New England Laminate	275
236. CMC-15 Bonding Film	Circuits Mat'l's Co.	275
237. Cat-A-Lac 643-1-1	Finch Paint & Chem	210
238. Number 4170 White Paint	Pettit Paint Co.	135
239. Number 803 Black Paint	Pettit Paint Co.	155
240. SC736928-I	Pettit Paint Co.	300
241. Space Guard Number 36231	Dexter Paint Co.	250
242. Moxness Compound MS50S14, Red	Moxness Rubber	300
243. C9-4183/H-3840	Hysol	330
244. XB-AR5R5-AXX Polyester Cloth	Ametek	135
245. DAP FS10	Allied Chemical	135
246. P422 Tape	Permacel	135
247. SR2702-75, Viton E60C	Stillman Seal	295
248. Scotchcast XR-5068	3M Co.	310
249. X-6700 TFE Bonding Film	3M Co.	310
250. Sylgard 187	Dow Corning	170
251. Scotchcast 243	3M Co.	135
252. Bondmaster E645	PPG	135
253. Eccofoam SH	Emerson & Cumming	210
254. Eppi-Bond 123/Cat 931	Furane	175
255. FM1044R/BR1009-49 Primer	American Cyanamid	135

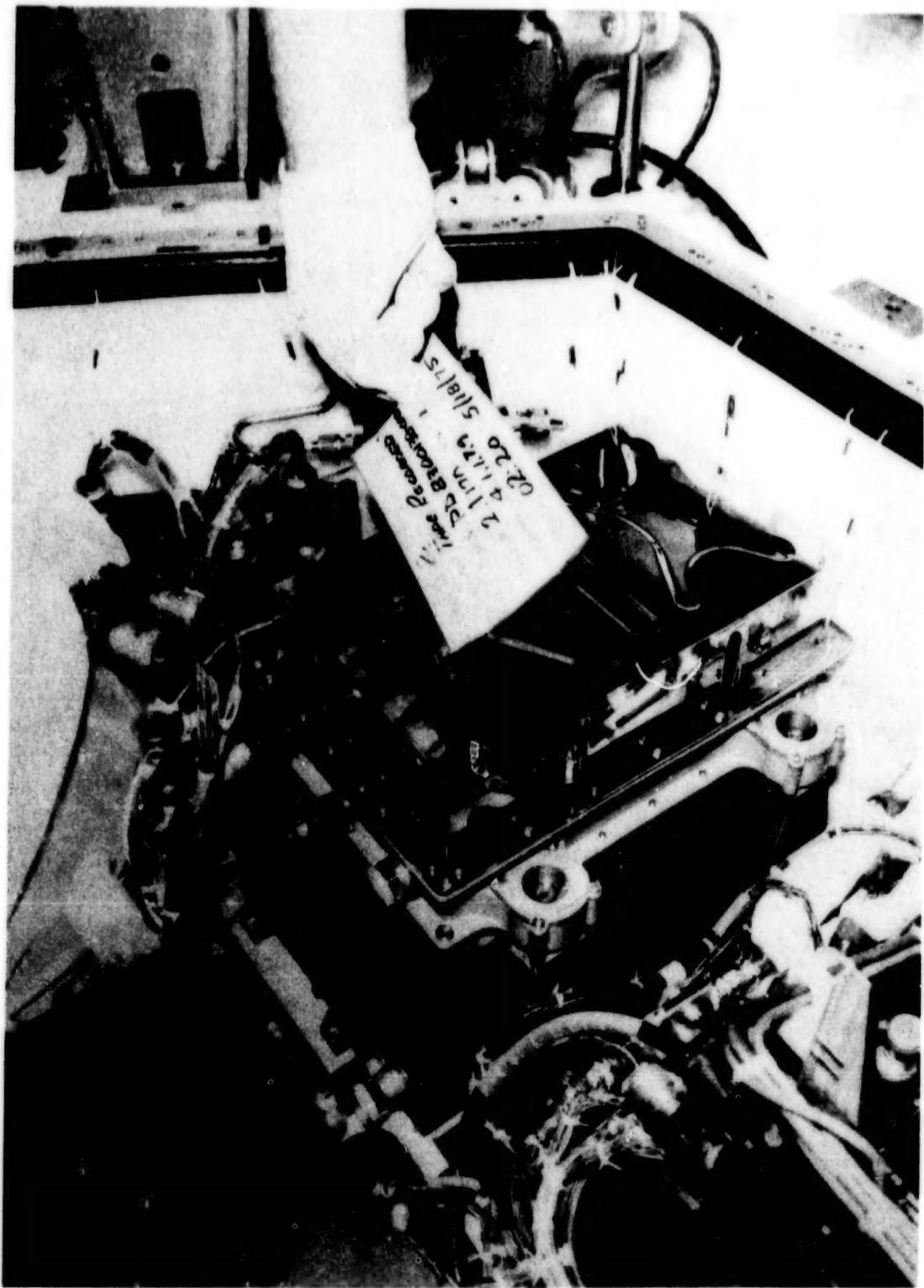


FIGURE 35. — Viking tape recorder assembly.

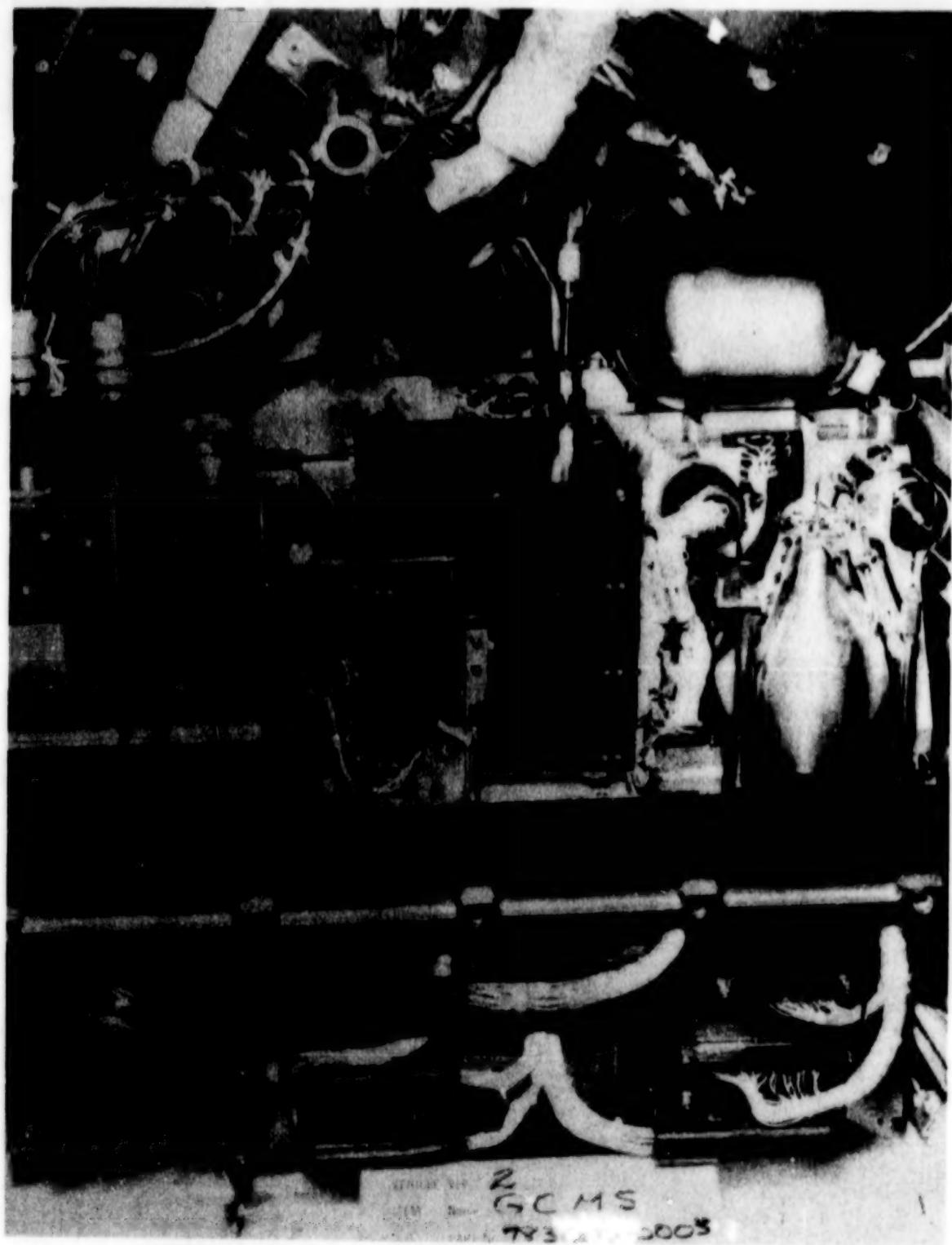


FIGURE 36. — Viking gas chromatograph mass spectrometer analysis instrument.

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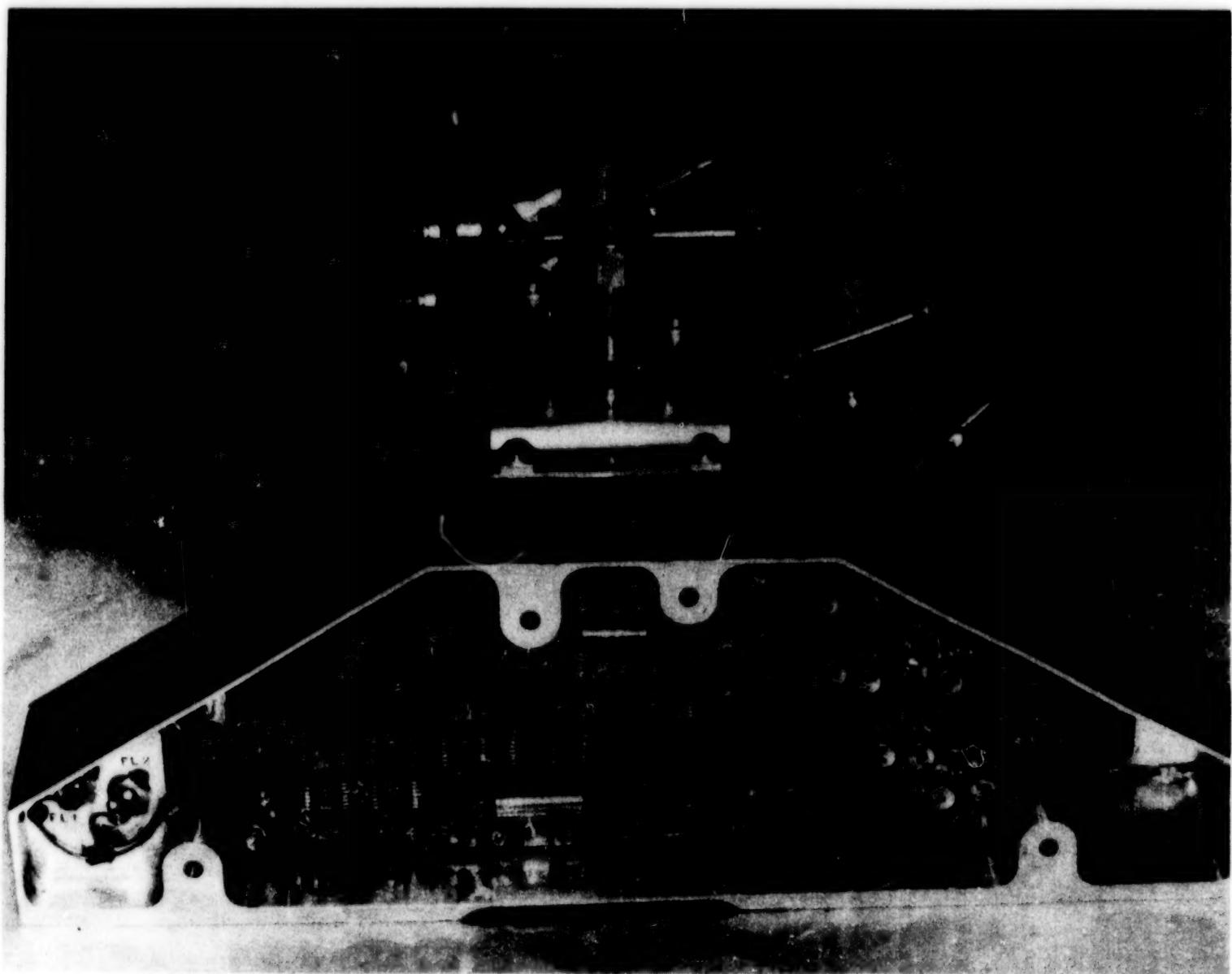


FIGURE 37. – Viking camera duster electronics assembly.

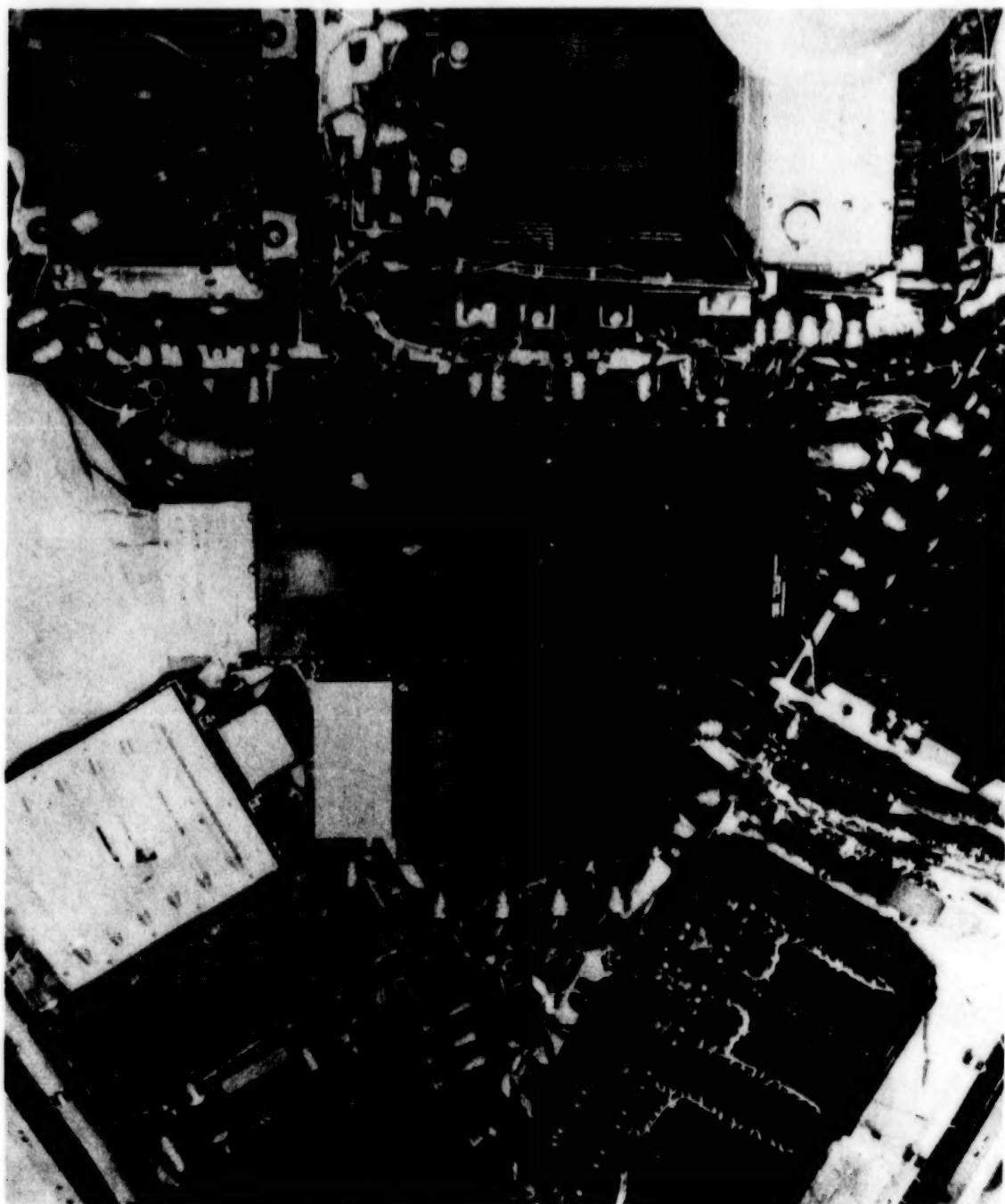


FIGURE 38. - Overall view of Viking electronic assemblies.

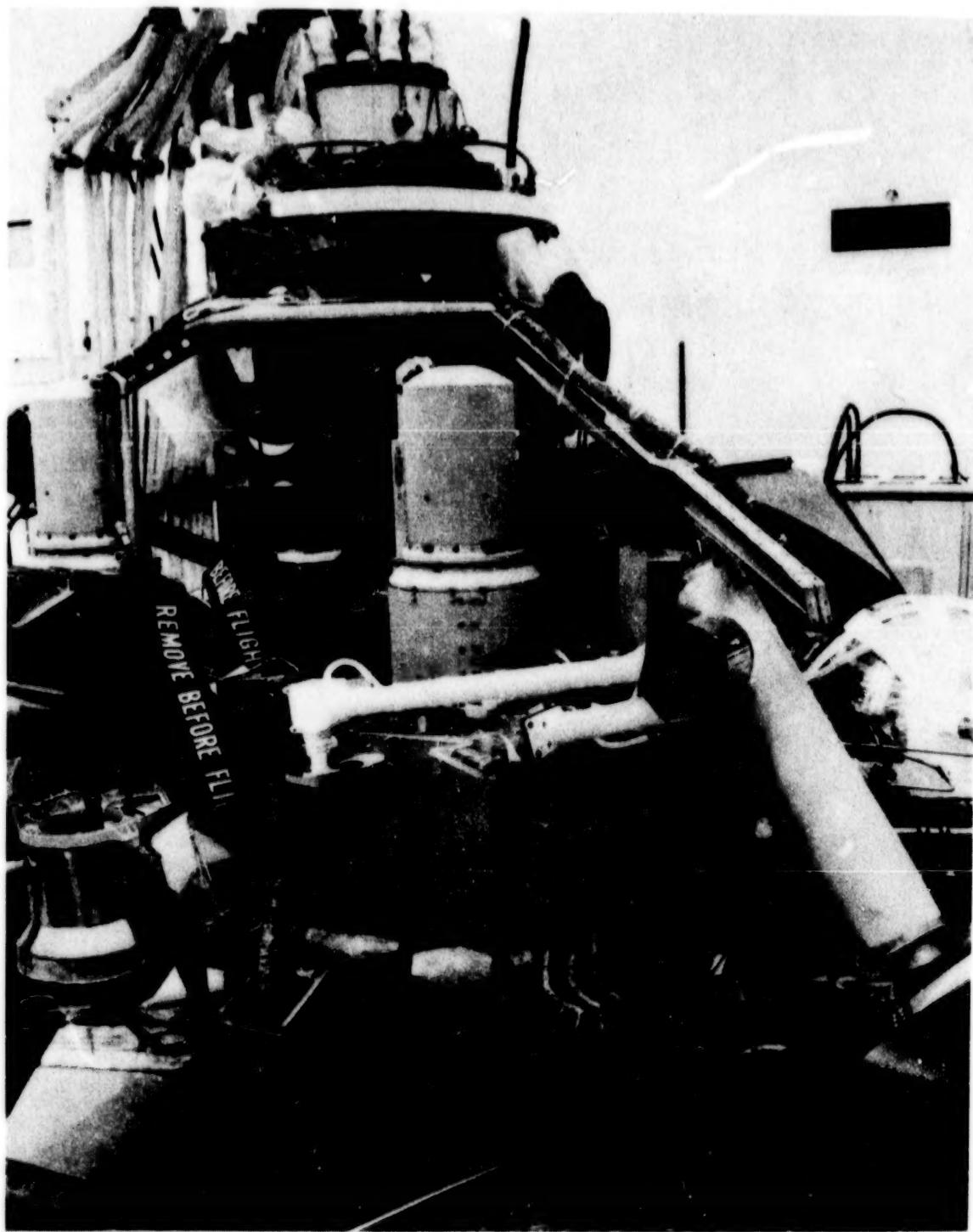


FIGURE 39. - Viking flight capsule prior to shroud assembly.

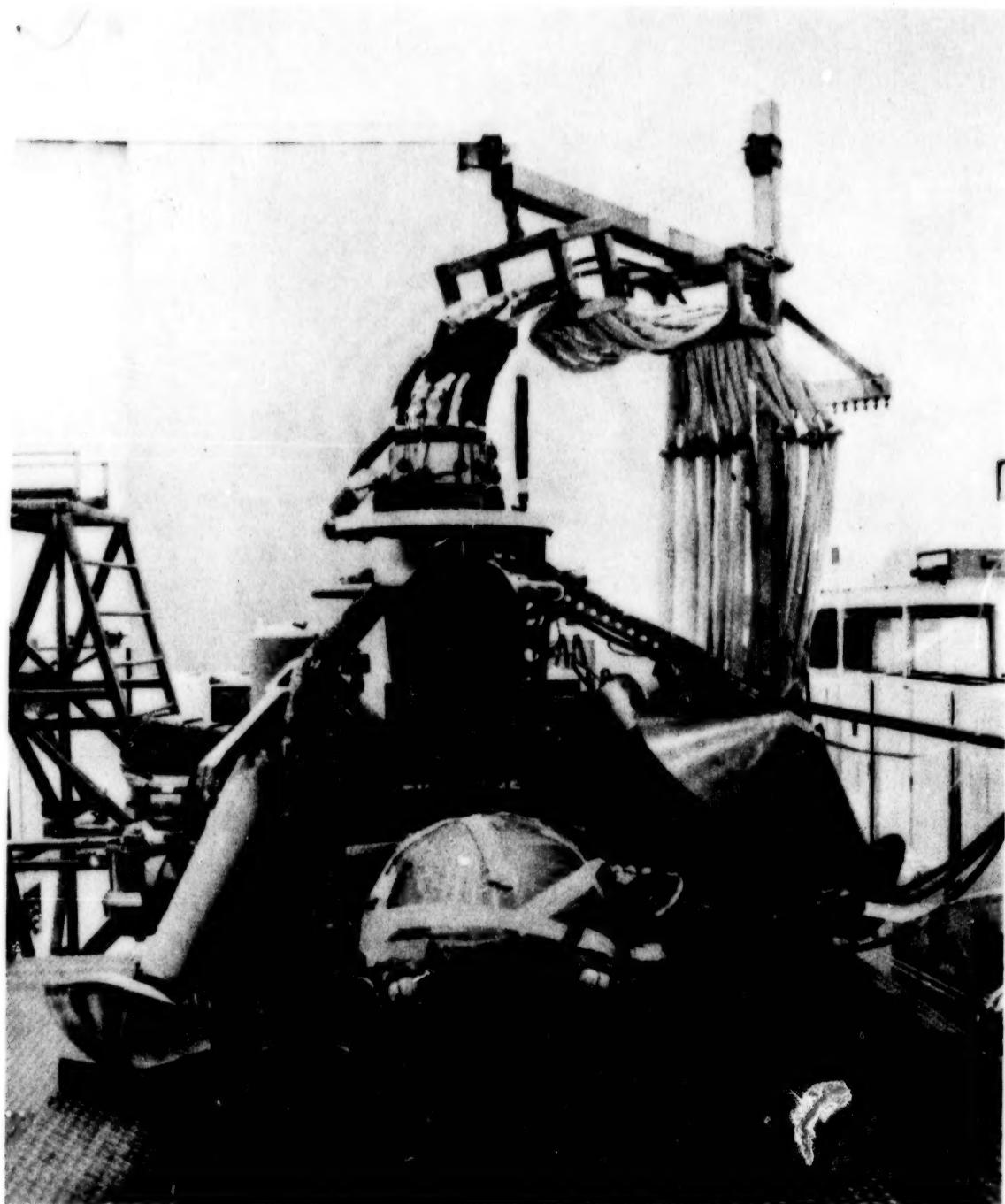


FIGURE 40. — Second view of Viking flight capsule 1.

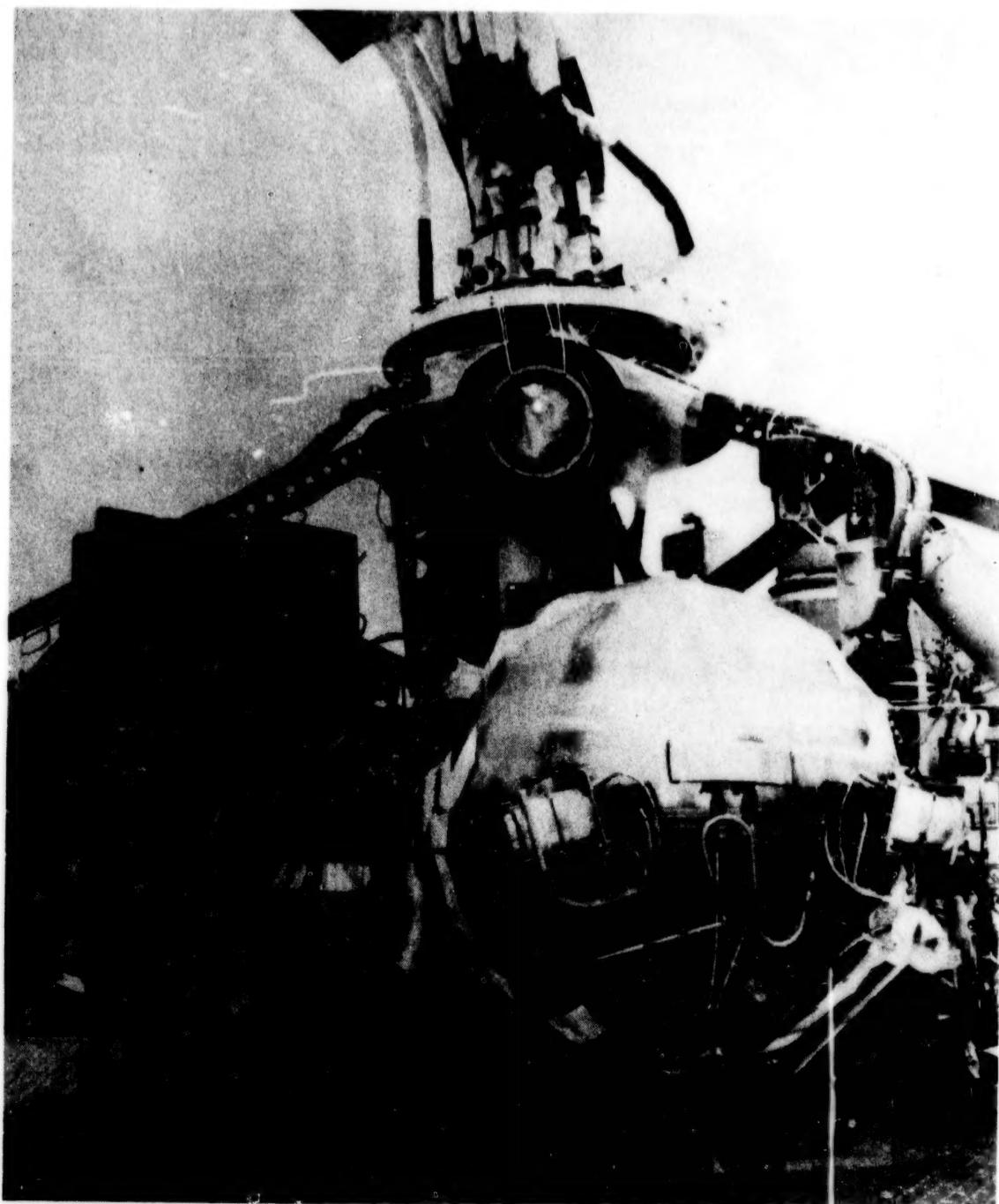


FIGURE 41. - Third view of Viking flight capsule 1.

Biological Barriers for Aseptic Assembly

*"They donned their rubber mittens and they took it
by the hand"*

INTRODUCTION

Despite the "friendly" and essential role micro-organisms play in the balance of terrestrial nature as we know it (they assist in decay processes, they convert insoluble protein to soluble protein, they work with higher plants in the fixation of nitrogen, and they convert sugars to alcohols), they also sometimes get in our way.

Previous chapters have discussed the various means of eradicating these "bothersome creatures." This chapter will discuss the biological barriers which NASA research has developed to keep them in or out.

Biological barriers such as human skin have been around for a long time, but are not usually recognized as such. A deep-sea diver's suit, a spacesuit, the rubber mittens in the poem, and window screens are common types of biological barriers.

Methods for microbial isolation were first developed in the 19th century. These ranged from hand washing to the development of pure culture and aseptic techniques in the latter part of the century. These techniques depended heavily on hand operations in which the skill of the worker was used to prevent sterile objects from coming into contact with contaminating materials; however, these classical methods did not suffice for the more exacting contamination control needs of space exploration (and modern medicine).

One of the first uses of a closed system as a biological barrier, you will recall from chapter 2, was the food canning process invented by Appert in Paris in 1810. Starting about 1885, workers interested in studying germ-free life developed many types of microbiological isolation systems. They used simple flasks and bell jars and eventually used modified bell-jar barrier systems in the early 20th century.

The concept of the sterile room derives primarily from germ-free research. Schottelius (1899) built

the first germ-free room in the center of a large empty room at the Institute of Hygiene of the University of Freiburg. This room was used in an attempt to raise germ-free chicks. One of the most elaborate, early barrier cabinets was devised by Kuster (1915) for rearing germ-free goats. It contained essentially all the features of modern-day biological barriers for germ-free animals, including an entrance airlock, air supply filters, and operation at a positive pressure. This apparatus was improved by later workers such as Glimstedt (1936) and Reyniers (1943). Reyniers (1959) also described a room-sized tank (2.5 meters in diameter and 5 meters long) used for rearing germ-free animals. Flexible plastic barriers, at a positive pressure for the absolute containment of germ-free animals, are largely a development of Trexler (1960).

Microbiologists handling infectious disease agents have long realized the need for biological barriers to internalize hazardous procedures. Safety cabinets were in use in German laboratories early in the century. In 1945, scientists at the National Institutes of Health developed a wooden cabinet for hazardous laboratory operations. About the same time, workers in England developed cabinets for use during large-scale production of scrub-typhus vaccine. The first stainless steel microbiological barriers used for work with infectious disease were described in 1953 by Wedum.

CHARACTERISTICS OF BIOLOGICAL BARRIERS

Classification

Biological barriers can be classified according to:

- Purpose—product protection or personnel protection
- Size—room size or cabinet size
- Degree of containment or exclusion—absolute barriers or partial barriers.

Tasks, such as spacecraft sterilization and aseptic filling of ampules, require product-protection systems. Personnel-protection barriers are used for research workers handling infectious microorganisms and for the containment of materials or samples returned from other planets.

Contaminating materials are separated from the worker by placing them in an enclosure or cabinet, or the worker is encased within an environment and separated by protective clothing. Therefore, it is possible to wrap a protective barrier around the work or the worker. For example, a worker in a clean room, wearing a sterile, plastic suit with separate air supply and exhaust lines, would be protected from external contamination. A lesser degree of worker isolation would be represented by a worker in a clean room wearing a respirator and sterilized garments. Hoods, cabinets, germ-free isolators, and similar enclosures around contaminating material are examples of barriers which protect personnel.

Absolute barriers allow no interchange of the protected and nonprotected environment and aim for total containment. They usually provide for placing the contaminating material within a gas-tight enclosure. Humans are separated from the material and the work is done by attached, arm-length rubber gloves or by remote mechanical manipulators. When materials within the barrier are to be protected from outside contamination, the enclosure is maintained at a positive air pressure. Conversely, negative pressure is used to prevent escape of contaminants. According to the criteria for biological control, ingoing and/or outgoing air may be filtered or heated to destroy microorganisms. Prior to use, the enclosure may be decontaminated or sterilized. Air locks, dunk baths, autoclaves, and other devices may be used to preserve the sterile integrity of the enclosure while materials are passed in and out of it.

Absolute containment can be achieved in a room-sized environment, but only by the use of servomechanisms or the wearing of ventilated suits or some other enclosure by persons entering the room. An absolute barrier room for sterile assembly work must be closed and sterilized prior to use. Suited personnel enter through a series of air locks in which the outside of the protective suit is sterilized with chemical agents.

A partial barrier provides less-than-absolute containment or isolation. Open-panel, ventilated cabinets and hoods; clean benches; specific pathogen-free, animal rearing facilities; bio-clean rooms; and laminar flow rooms are examples of partial barriers.

The partial barriers are not gas-tight and are not completely closed. Containment or isolation depends on an inward or outward flow of air through an open working panel, open glove ports, or through or across a room area. Both incoming and outgoing air may be filtered. A great variety of partial barriers, for both product protection and personnel protection, are in use today. Of particular significance has been the wide acceptance and use of barriers using the principle of laminar air flow which has greatly increased the efficiency of partial barriers.

The degree of biological control achieved by many partial barrier devices is adequate to meet the established criteria; however, they have several disadvantages compared to absolute barriers:

- Partial barrier systems are difficult to design to be fail-safe.
- Air flow patterns are easily disrupted by people and equipment in the path of the air.
- The rapid movement of people and equipment often exceeds the velocity of the air flow in the barrier system. Most partial barrier systems use air velocities between 15.2 and 45.7 linear meters (50 and 150 linear feet) per minute; however, a person walking at a speed of 3.2 kilometers (2 miles) per hour is moving at 53.6 meters (176 feet) per minute.

Desirable Features

Irrespective of the shape and material used for a biological barrier, there are certain desirable minimum features:

1. It must have proper air flow within the barrier. For an open-panel barrier, this means low turbulence of inward or outward airflow to prevent the entrance or escape of airborne particulates. The minimum air velocity is 30.5 meters (100 feet) per minute. For a closed, gas-tight barrier, this means operation at a positive or negative air pressure of 0.5 to 1.0 inch of water. Internal ventilation patterns for gas-tight barriers should be determined by the nature of the operation. Some procedures may

- require ventilation with inert gases, or accurate control of temperature and humidity within the barrier. The rate of contamination generation within the barrier is also an important consideration when selecting barrier ventilation systems.
2. It must provide an efficient means of sterilizing or decontaminating all interior surfaces of the barrier.
 3. It must have appropriate filters or incinerators for the air supply or exhaust or both.
 4. Adequate viewing panels must be set up between the operator and the operation.
 5. The internal surfaces must be resistant to chemical corrosion and be free of cracks and crevices that would interfere with sterilization and decontamination.
 6. Proper arrangements must be made for handling materials within the barrier. For absolute barriers, this usually means availability of attached, arm-length neoprene gloves. Open-front cabinets should have an available panel to close the unit during decontamination. A detachable front panel containing ports for arm-length gloves ideally serves both open and closed cabinets.
 7. Appropriate air locks, dunk baths, autoclaves, gas chambers, and other devices must be attached to gas-tight barriers to allow passage of essential supplies and materials.
 8. A sizeable and well-structured working space must be built within the barrier to minimize the need to transfer material in and out of the barrier before completion of an operation.
 9. Appropriate services, such as electricity, gas, vacuum, air, light, ultraviolet radiation, water, and drains, must be provided.

Many of the above features also apply to room-sized barriers.

Selection Factors

Selection of the appropriate biological barrier involves consideration of at least nine major factors:

- Degree of containment
- Purpose and size
- Reliability
- Compatibility with work materials
- Compatibility with decontaminants

- Expected life
- Cost per unit of work area
- Operating costs
- Maintenance costs.

In addition, there are other human-related factors that may be considered: comfort, lighting, flexibility, portability, modular construction, filter efficiency, ventilation patterns, etc.

Cost data on room-sized, microbiological barriers are not readily established or compared because they vary so widely in design and purpose. Germ-free isolators made of unsupported plastic film represent the smallest, initial cost investment for small biological enclosures. For many applications, such as the rearing of germ-free animals, this type of barrier is preferred. However, easy rupture of the film, aging of the plastic under ultraviolet irradiation, lack of good viewing capability, seepage of gas through the plastic, and other similar factors may limit its usefulness for other applications.

NASA'S INTERESTS IN BIOLOGICAL BARRIERS

NASA's use of and involvement in the testing and development of biological barriers derives from both generalized and specific needs. The general need is based on operating philosophies, research and development modalities, and engineering and design requirements that permeate most of the Agency's activities.

In other words, very little of NASA's space flight program could be accomplished without some involvement of equipment and techniques to control contamination and increase system reliability.

Two specific interests of NASA in biological barriers have been spurred by requirements of the planetary quarantine program.

This program initially required the prevention of back contamination, that is, contamination brought back to Earth by extraterrestrial organisms. To accomplish this, NASA designed and built the Lunar Receiving Laboratory in 1967. This Earth-based NASA facility served as the temporary living quarters of returned astronauts. They remained there under quarantine until it was verified that they were free of harmful foreign organisms. The laboratory also was originally used to isolate and analyze lunar samples (figure 42). The sample analysis and crew reception areas were enclosed within specially built biobarriers.

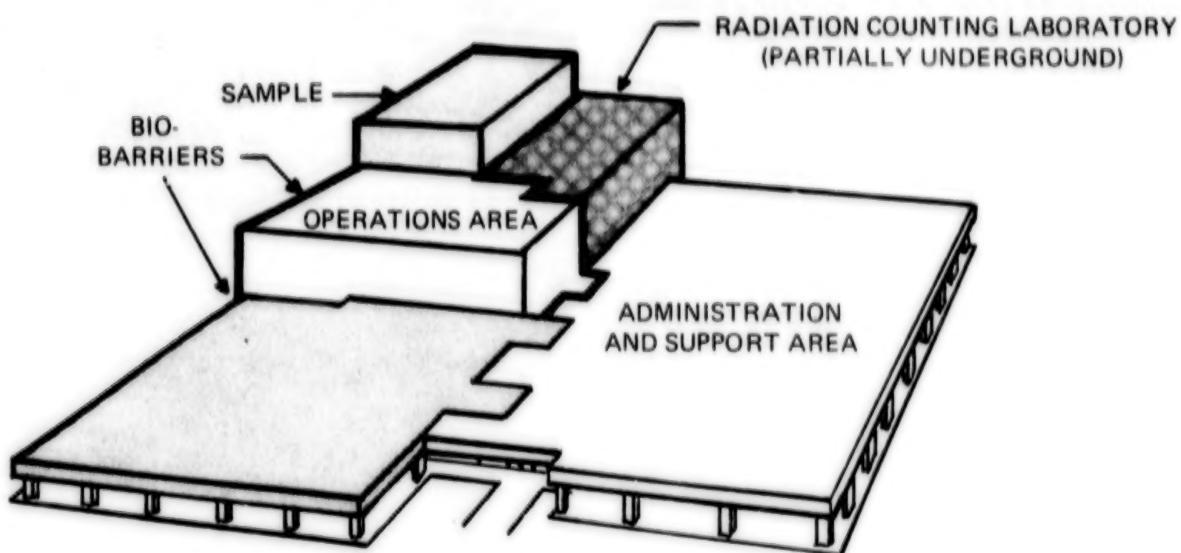


FIGURE 42. — Lunar receiving laboratory functional areas.

The most sophisticated microbiological barrier techniques were used to ensure that lunar samples did not carry contaminating organisms nor were contaminated by the experimenters.

The second requirement was the production of sterile planetary landers for the exploration of other planets to prevent their contamination by earthly organisms. For this purpose NASA investigated the feasibility of assembling a spacecraft from sterilized parts in a sterile environment to reduce the potentially deleterious effects of the terminal heat process (mentioned in earlier chapters). This technique is known as the "aseptic" or "sterile assembly" approach.

Specific NASA research programs involving biological barriers have ranged from the glove box assembly of sterile miniature electronic devices (figure 43) to the development of a full-sized facility for the sterile assembly of a complete spacecraft. Techniques were developed for the repair of sterile assemblies and for the insertion of presterilized components into spacecraft without violating the sterility of the assembly.

Because of its inherent complexity and cost, the concept of sterile assembly and sterile component insertion has not been extensively used by NASA for sterile flight hardware. The experience gained,

however, both in the Lunar Receiving Laboratory and in the sterile assembly investigations, suggests a number of potential nonaerospace applications:

- Air pollution
- Cancer research
- Food industry
- Fuel industry
- Hospital equipment and medical techniques
- Infectious disease research
- Optics industry
- Paper industry
- Pharmaceutical industry
- Photographic industry
- Product deterioration
- Water resources.

Early Investigations

Early NASA studies with biological barriers involved the use of isolation cabinets with attached arm-length, rubber gloves. These cabinets or isolators (figure 44) were designed so that the contents could be observed and manipulated and so that the materials could be introduced and removed without destroying the biological barrier. Cabinets have been made from a wide variety of materials, including stainless steel and plastics. Such cabinets are suitable for many



FIGURE 43. — The microbiological assay of spacecraft electronic components in a glove box.

scientific applications and for a variety of production purposes, but they proved unsuitable for the more rigorous aerospace applications for which maintenance of sterility during complex operations is required. NASA's procedures for the sterile assembly of electronic parts, for example, are very exacting. In addition, the mere size of spacecraft components prohibits the ready use of absolute barrier cabinets.

One of the early investigations, using sterile assembly techniques, evaluated the ability of personnel to perform certain assembly tasks in an

isolation cabinet (ref. 182). Trained people from a telephone equipment assembly line were used. They performed standard assembly operations in a flexible film isolator. The results indicated the following:

- There was no significant difference in assembly time when surgical gloves were used.
- There were no major operator complaints or discomfort.
- No visible damage was done to the flexible, plastic glove box after the soldering, cutting,

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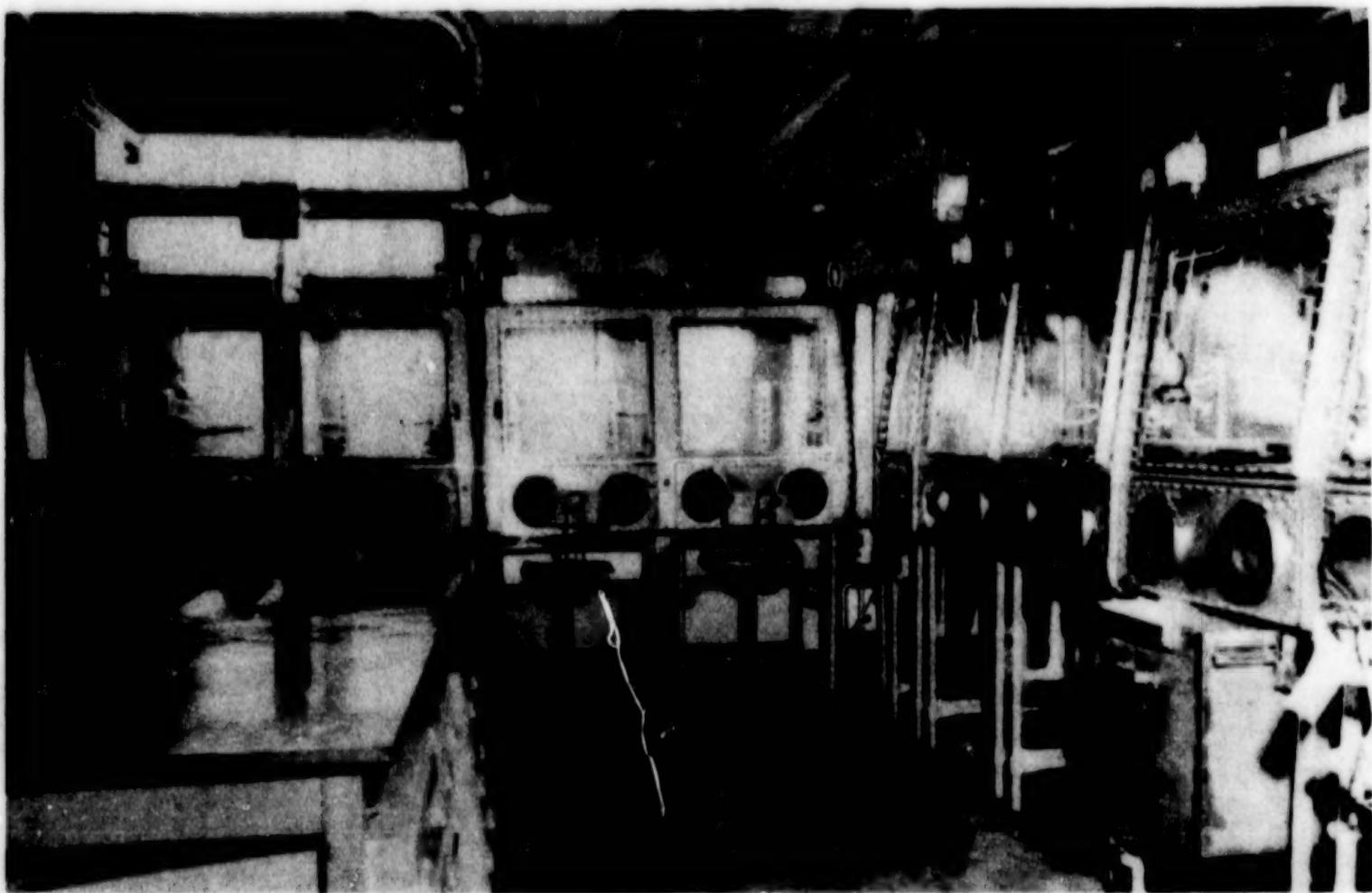


FIGURE 44. - Gas-tight modular ventilated cabinet system.

- splicing, and other manipulative assembly operations.
- There was no apparent quality difference between completed assemblies from the production line and from the glove box line.

While this study was concerned primarily with the feasibility and functional aspects of sterile assembly, it was also necessary to demonstrate the reliability of the technique for maintaining sterility.

It had to be shown that sterile parts could be transferred into a sterile cabinet without compromising the sterility of either one. Sterile components had to be assembled within the cabinet with the confidence that the end product would not be contaminated. Finally, since an inadvertent small puncture of the gloves or of the cabinet walls was a potential source of undetected contamination, a means for detecting such punctures was required. Consideration of these problems required the sterility testing of such large numbers of samples that the expense of time and cost ruled out the use of cabinets for sterile assembly of spacecraft. Nevertheless, some ingenious approaches were developed to establish a better statistical basis to assess and assure that sterile conditions for parts transfer and assembly were maintained.

Sterile Parts Transfer

To determine if sterile parts could be transferred into sterile cabinets without contaminating either one, a test assembly (figure 45), which used a cabinet within a cabinet (ref. 183), was developed. The intervening environment contained a high concentration of airborne *B. subtilis* var. *niger* spores. This high challenge rate provided the statistical basis for establishing the needed confidence in the technique.

The exterior, plastic film cabinet 3.35 m by 1.67 m by 1.53 m (11 ft by 5½ ft by 5 ft) was placed approximately 1 meter from the laboratory floor to permit personnel to enter the captive suit (built as an integral part of sterile lock isolation unit) from the bottom. A nebulizer and fan were used to maintain an airborne concentration of approximately 10^6 spores per cubic foot of air. Prior to operational use, the interior of the sterile lock was sterilized with a mixture of 88 percent ETO and 12 percent Freon-12 and then purged with sterile air.

Personnel wearing the captive suit in the exterior cabinet transferred electronic parts from that cabinet

isolator through the contaminated atmosphere and into the sterile lock port. The ETO mixture was then introduced into the port to sterilize the parts. After sterilization, the operator entered the captive suit of the sterile lock cabinet, removed the electronic parts from the port and performed a simulated assembly operation.

Three biological measurements were made within the inner cabinet to detect evidence of contamination: (1) the inner walls were swabbed to collect organisms deposited on surfaces, (2) a liquid impinger air sample was used to determine the presence of airborne contamination, and (3) the parts were incubated in sterile broth to detect any contamination.

Twenty-three sterile lock transfers were made with the ETO mixture as the sterilant. Twenty were germ-free, and three had an accountable source of contamination. In addition, three peracetic acid transfers were made with complete success. The results indicated that successful transfer could be accomplished, and later confirmation was provided by other studies that used a peracetic dunk bath and pass-through autoclaves (ref. 183).

Sterile Assembly

Having demonstrated that sterile parts could be successfully passed into a glove box, it remained to be shown that the assembly operations could be performed to provide a sterile product under glove-box conditions. This was proven by several investigations. One study examined the effect of internal atmospheres of sterile air, sterile nitrogen, and pure ethylene oxide on a variety of standard assembly procedures, including soldering, bonding, and nut-bolt manipulations (ref. 184). The performance and reliability of the assembly were then evaluated.

An oscillator circuit was used as an experimental electronic assembly. The cabinet arrangement consisted of a main cabinet 1.83 m by 1.22 m by 1.22 m (6 ft by 4 ft by 4 ft) with 4 work stations where the assembly operations were conducted, an interchange lock connected to the main cabinet, an initial storage location for the electronic parts and tools, and an entry lock connected directly to the main cabinet (figure 46).

The atmosphere of the main cabinet was maintained by use of a sterilization cart with canisters of ETO, sterile nitrogen, and sterile air attached to inlets on the cabinet. Other features of the main

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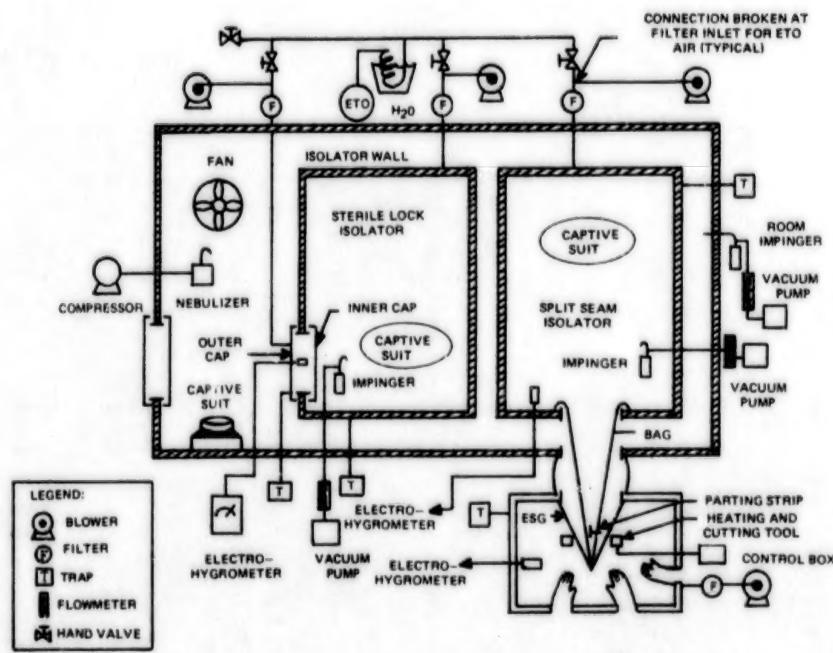


FIGURE 45. – Schematic of insertion apparatus.

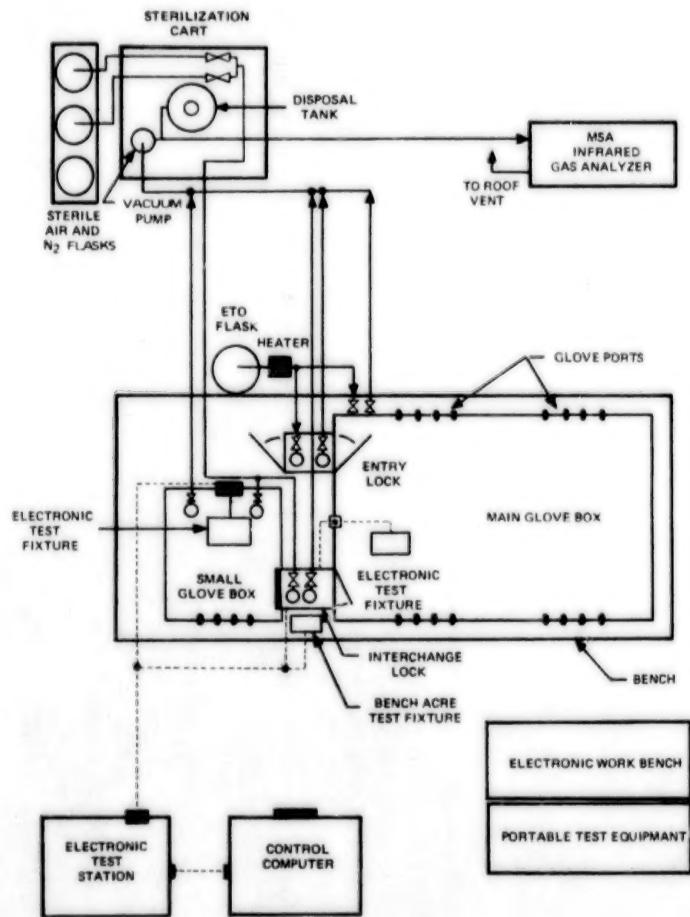


FIGURE 46. – Sterilization laboratory layout with major system interconnections.

cabinet set-up included: (1) a flexible, plastic top to accommodate pressure fluctuations resulting from the insertion of arms into the gloves, (2) an emergency blow-out if the overpressure appreciably exceeded the intended maximum internal pressure differential of 1245 N/m² (5 in. of water), (3) an inflatable rubber seal around the entry door, and (4) an inlet plenum with baffles to reduce turbulence during filling of the box with gases. Butyl rubber (Buta-Sol^R) gloves of 0.076 cm (0.03 in.) thickness were used in the tests and these showed no evidence of ETO leakage. The study showed:

- Assembly of electronic circuits under sterile atmospheres of ETO/Freon-12, sterile air, or sterile nitrogen was feasible with the cabinet techniques.
- Performance of the assembled unit was not affected by its assembly in a cabinet containing atmosphere of either ETO/Freon-12, sterile nitrogen or sterile air.
- Hand soldering, dip soldering, staking, nut-bolt assembly, epoxy bonding, and potting were not harmfully affected by ETO/Freon-12 atmosphere.
- Cabinet assembly could increase the time required to achieve the same product quality by a factor of 3.

In another study, silver-zinc batteries were selected as the test article because of their heat sensitivity and their importance as a spacecraft power source (ref. 185). The investigation included the sterile assembly in a cabinet of the batteries of sterilized parts. The results were most encouraging. Glove-box assembled batteries showed appreciably less degradation in electrical performance tests than did normally assembled batteries exposed to heat sterilization after assembly. No biological contamination was detected during the assembly operation.

In other studies NASA used two specially designed glove box systems of a sterile access system for the post-terminal sterilization, adjustment, and repair of spacecraft: the glove box assembly sterilizer system (GBASS) (ref. 186) and the pilot assembly sterilizer system (PASS) (ref. 187).

The Puncture Problem

A large hole or tear in the walls of a flexible film isolator or in the gloves in any glove box can be seen and corrected easily, but a small puncture could occur and not be detected for some time. Two

approaches were taken to solve this problem. The first was an attempt to develop a method for the rapid detection of punctures should they occur. The second approach was to study techniques that might be used to overcome puncture hazards.

Developing a method for the rapid detection of contamination by a puncture was problematic. The usual techniques for detecting microorganisms require use of a growth media for a period of time which was longer than acceptable; therefore, traditional culture techniques could not provide a practical monitoring tool. However, the problem of detecting punctures was solved by the development of an indirect alarm system that provided the necessary short reaction time (ref. 184).

A double-walled, aseptic assembly facility was designed as a series of connected cabinets. An aerosol of microscopic, fluorescein particles was dispersed between the double walls at a pressure above both the internal pressure of the cabinets and the external room pressure. Thus, a leak in either wall would carry the aerosol from the sterile interstitial walls into either the inner cabinet or the room. Particles escaping through these holes were detected by a sensitive optical device measuring fluorescence. The surgical gloves that were used were also double-walled, so that the aerosol was present between the glove walls as well.

Using this system, many types of spacecraft hardware were successfully assembled and were found to remain sterile. When punctures were deliberately produced, the optical device quickly detected the presence of the fluorescein particles and the alarm bells sounded. Even so, other problems were uncovered during use of the system: (1) the aerosolized particles tended to precipitate into the plenum between the chamber walls after long-term use; (2) the accumulation of thick particle deposits in the double-walled gloves required that they be changed frequently, thereby increasing facility downtime; and (3) the double-walled gloves reduced the manual sensitivity of the operators, so that extra time was required for training and practice. While not ideal, the system was used to accomplish sterile assembly while ensuring puncture detection.

The Flow of Bacteria Through a Puncture

A review of the problem of contamination caused by small punctures in cabinet walls raised the

question of whether bacteria could swim upstream. Many microorganisms are mobile. They can move about freely in drops of water, propelled by the movement of hairlike structures (flagella or cilia) exterior to their cell walls. In a dry environment such as in the air of a spacecraft assembly area or on the walls of a cabinet, moisture is specifically excluded because of its deleterious effect on the metallic parts being assembled. Therefore, it was questioned whether or not bacteria exterior to a glove box—in the absence of moisture and with positive pressure within the glove box—could move against the flow of gas, through a small hole, and penetrate into the

working area. Positive pressure differentials were commonly used to reduce the probability of contamination in clean rooms and cabinets used for gnotobiotic work; however, insufficient quantitative data were available to state confidently that such positive pressure would insure that contamination transfer would not occur.

A series of investigations were performed to provide the necessary data. The first study (ref. 183) used the test apparatus shown in figure 47. The study was designed to evaluate the flow problem through circular holes varying from 0.32 cm to 1.9 cm (3/4 in.) in diameter. The adjacent, enclosed

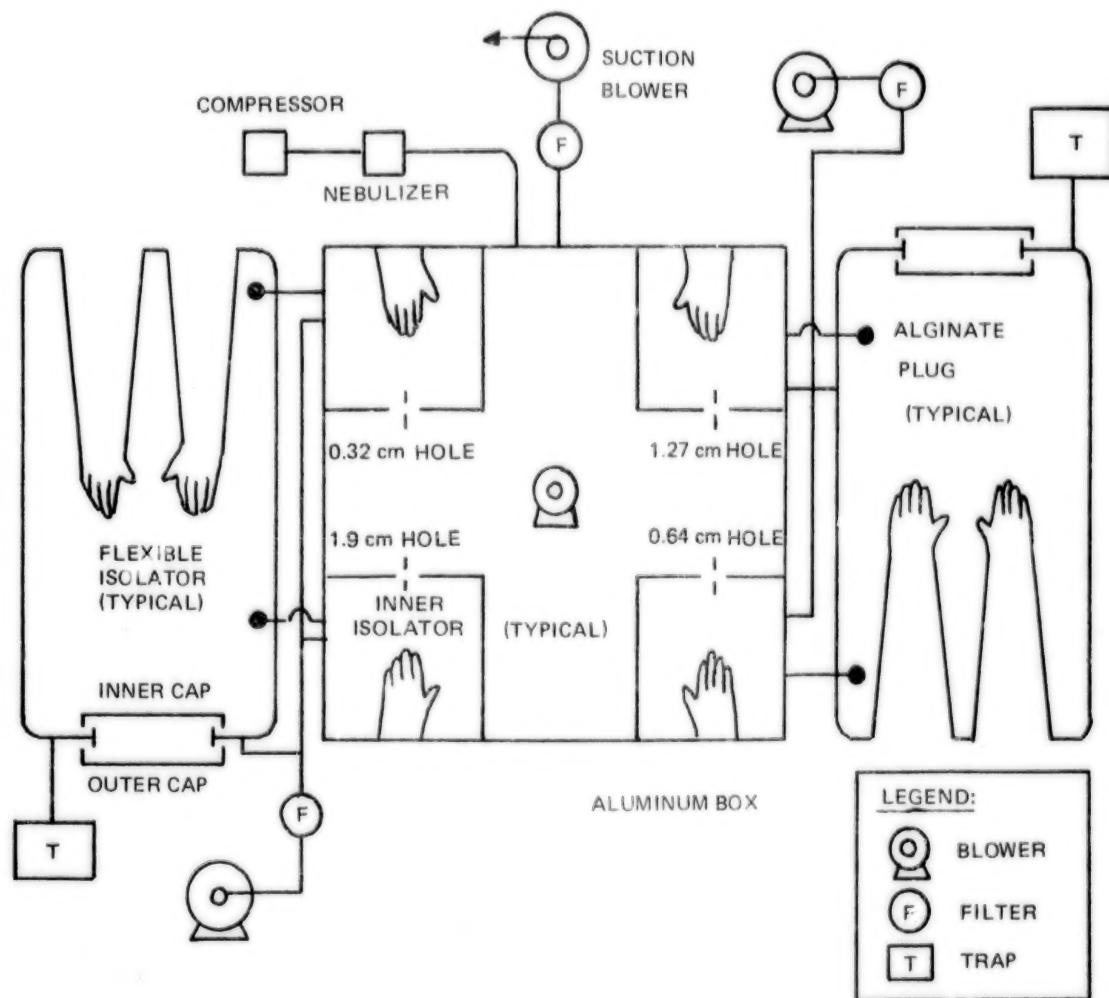


FIGURE 47. — Schematic drawing of test apparatus for leakage through small hole.

region contained approximately 10^5 spores of *B. subtilis* var. *niger* per cubic foot to assure a highly contaminated environment. The pressure differential across the holes of the cabinets varied from 25 N/m^2 (0.10 in. of water) for the 1.9 cm diameter hole to 62.5 N/m^2 (0.25 in. of water) for the 0.32 cm diameter hole. Assays were taken daily and no evidence of contamination was found over a continuous, 3-week test period.

These data proved the feasibility of the approach, but were not considered sufficient to justify the use of a pressure differential as an acceptable safeguard against contamination. A specific concern was whether the turbulent flow from holes of irregular shape would be as effective.

Additional investigations evaluated particle movement against pressure differentials under conditions in which the particles were initially in a quiescent state and then were in a moving stream impinging on the hole.

An experimental arrangement developed for the quiescent studies is shown in figure 48. Lyophilized (freeze-dried) microorganisms were aerosolized through the dispenser head into a 22-liter, spherical glass flask. The agitating fan was used only momentarily (the hole was covered during this period) to circulate the organisms and was then shut off. The dispersed organisms were then allowed to challenge the hole in the membrane against the pressure differential. Membranes provided with a variety of test hole sizes were examined. In addition, adjustment of the gas supply (including zero for the condition of no-pressure differential) provided a range of pressures. The cylindrical culture chamber permitted the definite location of contamination. Even more important, it permitted a quantitative measure of the number of organisms that penetrated the hole; i.e., from the number of colonies that later developed. The filters assured sterility of the pressurizing gas.

The quiescent test results were consistent with the theoretical analyses. For hole sizes of 1000×10^{-6} to 3000×10^{-6} m (1000 to 3000 microns) no contamination was detected for pressure differentials of 12.55 N/m^2 to 125 N/m^2 (0.05 in. to 0.50 in. of water). From these data, a conservative value of 125 N/m^2 (0.05 in. of water) differential pressure can be considered to prevent the penetration of bacteria (through holes in the size range) under quiescent conditions.

The moving-stream studies were conducted in three wind tunnels using the test set-up in figure 49. Fifty tests were run. Hole sizes of 20×10^{-6} to 1000×10^{-6} m (20 to 1000 microns) in diameter, and pressure differentials ranging from 12.5 N/m^2 to 950 N/m^2 (0.05 to 3.8 in. of water) were used. Wind velocities varied from 16 to 48 km/hr (10 to 30 mph). The results of holes of 20×10^{-6} , 200×10^{-6} and 1000×10^{-6} m (20, 200, and 1000 microns) indicated critical velocities of 48, 41, and 24 km/hr (30, 26, and 15 mph) respectively. As the velocity increased beyond these points, a marked increase in pressure differential was required to prevent particle penetration.

Another problem encountered was the estimate of the time delay before contamination occurred, once the pressure differential was lost. The quiescent apparatus was used (figure 48). The pressure differential was controlled at 500 N/m^2 (2 in. of water) for a given time, then the pressure was vented to zero for a specific time and then returned to 500 N/m^2 . Experimental tests on 100×10^{-6} , 200×10^{-6} , and 1000×10^{-6} m (100, 200, and 1000 micron) holes showed minimum contamination times of approximately 1 second. For the smallest hole tested, 20×10^{-6} m (20 microns), 30 to 60 seconds were necessary for contamination—the smaller hole size permitted less frequent challenge by viable particles.

These data showed that a high probability of sterile conditions could be assured when a positive internal pressure was used to preclude microbial penetration of small holes. NASA has adopted the conservative approach that the maintenance of a pressure differential of 1245 N/m^2 (5 in. of water) is sufficient to prevent contamination through small holes. The Viking Project maintained this pressure differential within the bioshield, which encapsulated the Mars lander, to assure that it was not recontaminated after sterilization and prior to launch.

The potential for accidental transfer of organisms through any biological barrier has different implications depending on the nature of the task. When potentially dangerous organisms are handled, precautions must be taken to protect the operator and his environment from contamination if the biobarrier is violated. In this case, the external pressure should be greater than the internal pressure to retain the organisms within the biobarrier. Conversely, when the intent is to maintain sterility of an object, a greater internal pressure should be used to prevent the ingress of undesirable organisms.

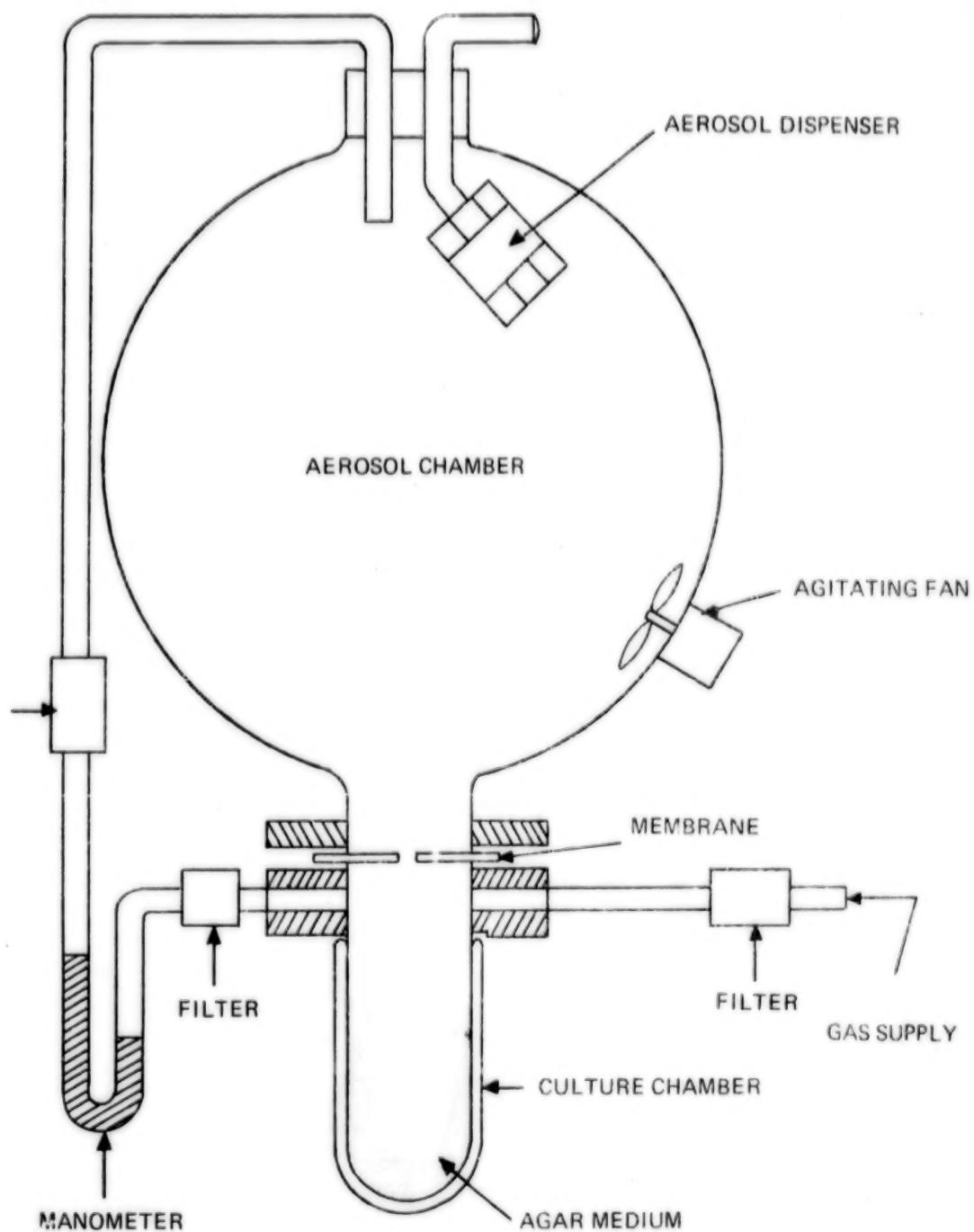


FIGURE 48. — Experimental apparatus, quiescent state.

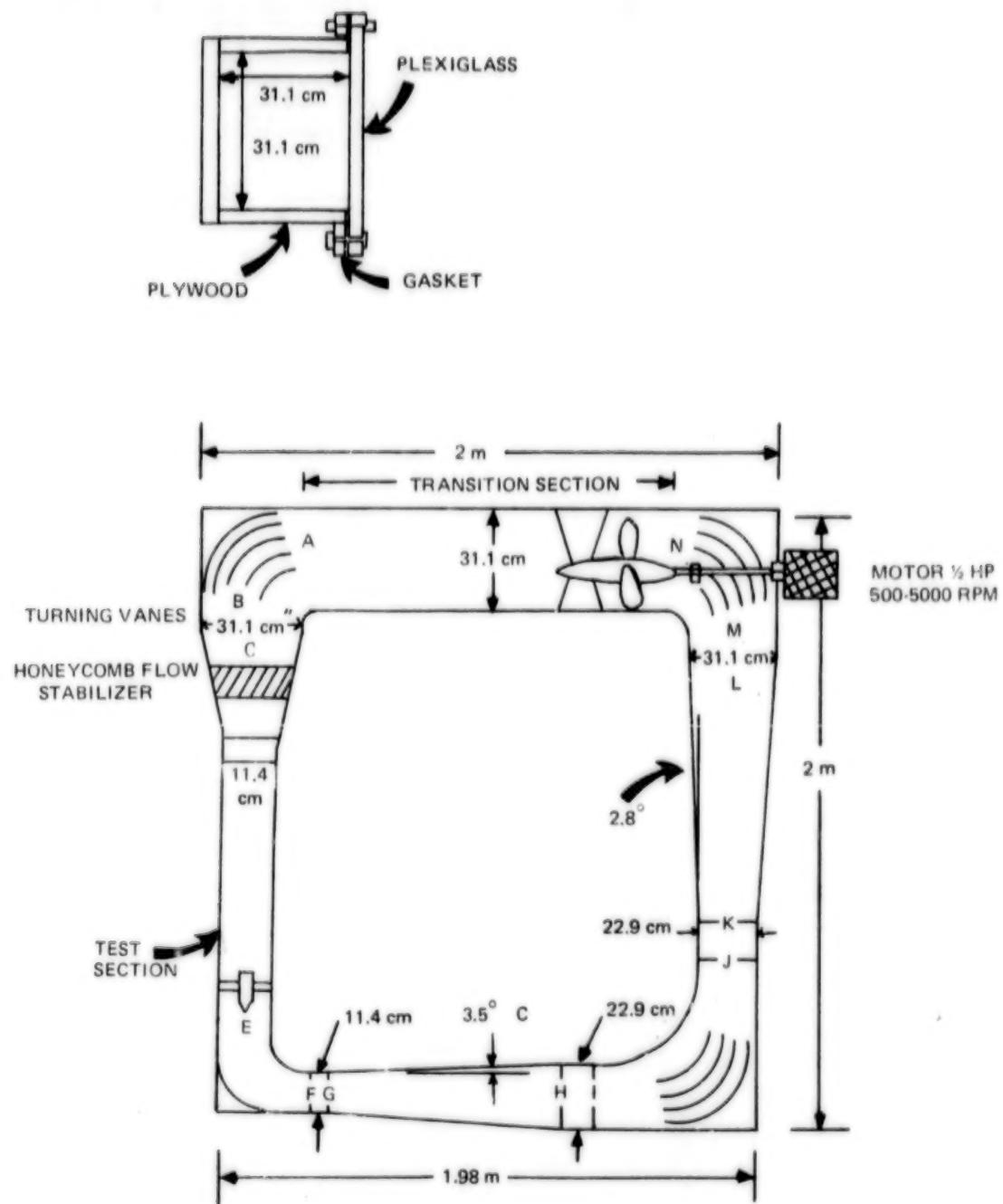


FIGURE 49. – Flow chamber design (high speed).

Summary of Results

The NASA investigations on the use of absolute barrier cabinets for sterile assembly had significant results:

- It was shown that sterile electronic components could be reliably assembled using complex and delicate operations under glove-box conditions.
- A pressure differential of 1245 N/m^2 (5 in. of water) was proven sufficient to negate the problem of contamination through small holes if hole size does not exceed 1000 micrometers and air velocity does not exceed 48.27 km/h (30 mph).
- A double-walled chamber and a technique that permits rapid detection of punctures were developed.

NASA's investigations did not stop here. The absolute barrier cabinet concept was expanded to a full facility concept. Men in barrier suits replaced the hands in gloves, and now an entire spacecraft can be assembled from sterile components in a sterile environment.

EXPANDED ABSOLUTE BARRIER CONCEPT

A program was initiated to determine the feasibility of a full-scale, sterile assembly facility—one large enough to permit assembly of a complete spacecraft. First an analog of the system was designed and fabricated (ref. 188). This system is shown in figure 50. Operations simulating spacecraft assembly and repair, including adhesive bonding, riveting, and screw-attachment activities, were conducted in the unit.

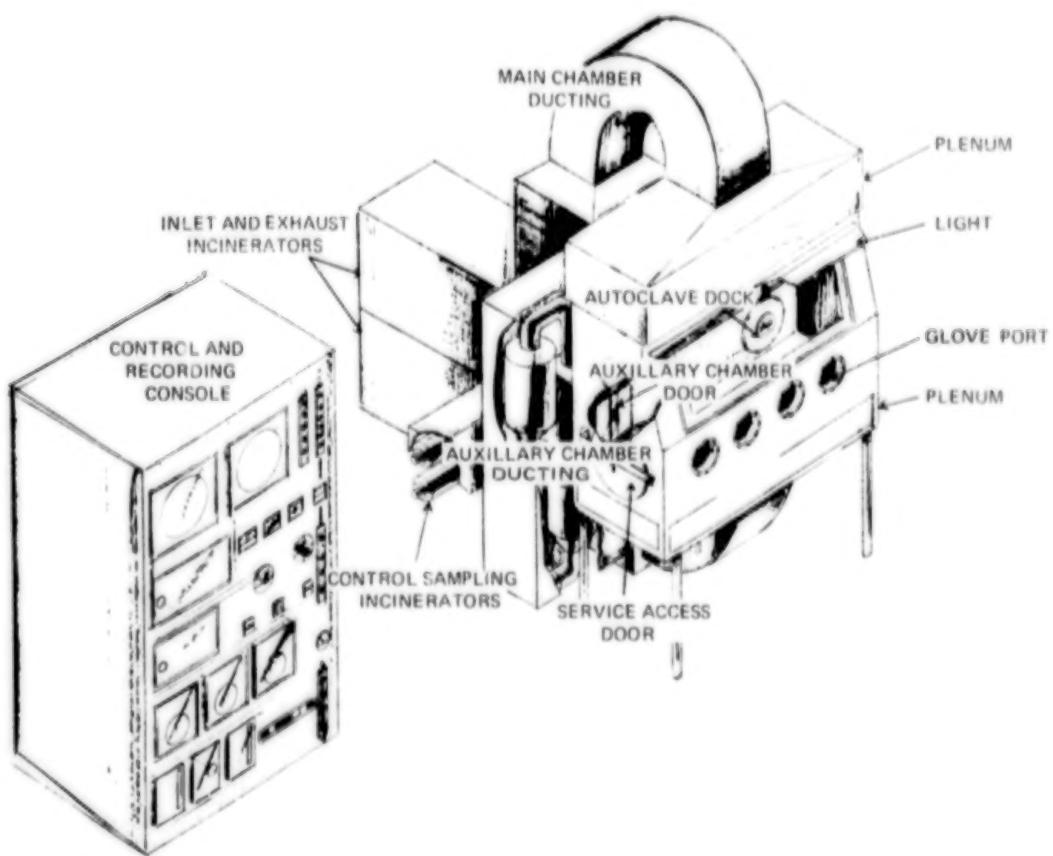


FIGURE 50. -- Conceptual drawing of assembly analog.

A limited, human-factors test program was also conducted to determine operator restrictions such as discomfort and the reduction in manual dexterity. Human operators, if carefully selected, were found competent to perform fairly complex operations with little discomfort. Tool constraints were evaluated and certain tools, such as screwdrivers with plastic handles and those containing moving parts, were found to be incompatible with sterile conditions; however, only minor modifications were required to resolve these problems.

The biological assay activities of this analog project provided measures of bioburden, sterilization verification for the chamber, and packaged and unpackaged test specimens. The assays showed the analog to be able to maintain sterility during the simulated operations.

The results of the analog work suggested the feasibility of a full-scale, sterilizing assembly. Based on these results, a full-scale mobile prototype termed MAST (model assembly sterilizer for testing) was designed, fabricated, and tested. Concurrent with the analog activity, NASA also developed and demonstrated the feasibility of a total biological barrier suit and, subsequently, developed a biological isolator suit system (BISS) for MAST.

MAST

The MAST, presently located at the University of Wisconsin, is shown in figures 51 and 52 (ref. 189). This assembly is a three-trailer complex, designed to permit sterilization and sterile assembly of a space-craft 2.25 meters (7.5 ft) in diameter. MAST has the capability of performing most methods of decontamination or sterilization, including dry-heat sterilization, ETO decontamination, and steam autoclaving. The main chamber can be sterilized and it has facilities for Class 100 clean room operations (to be discussed in the second part of this chapter). During operation, the internal pressure is maintained above ambient pressure to minimize the possibility of organisms entering the chamber. MAST contains all the equipment and facilities required for operation, including boilers, refrigeration, pressurization units, vacuum systems, controls, indicators, and recorders. All MAST operations are directed from the control console exterior to the biobarrier. Continuous observation of activities within the biobarrier is also maintained from the console.

The MAST biological enclosure includes a 3-m by 6-m (10 ft by 20 ft) main chamber, in which major sterilization activities are conducted. Contiguous to the main chamber is the antechamber, which is a cylinder 2.4 m (8 ft) long and 2.4 m (8 ft) in diameter. The antechamber can be regulated from ambient temperature to 172°C. It has several modes of operation, including a vacuum cycle, an ETO/Freon-12 decontamination cycle, and a thermal sterilization cycle. Prior to entering the main chamber, large items (up to 2.25 m (7.5 ft) in diameter) are passed through the antechamber, where they are sterilized or decontaminated. A separate ETO/Freon-12, dry-heat pass-through is used for most sterile insertion transfers.

Biological Isolator Suit

Probably the most complex and innovative aspect of the MAST is BISS, which is the key to the MAST concept (ref. 190). BISS (figure 53) consists of a sealed, flexible suit coupled with a 3.2-m (15 ft) flexible tunnel, both of which are impervious to organisms. It allows a man to enter the sterilized chamber without recontaminating the sterile environment. One end of the tunnel is attached and sealed to the back of the suit and the other is sealed leak-tight to a door in the chamber wall. BISS then becomes an extension of the sterile chamber wall. The operator within the suit is isolated from the chamber environment. The tunnel provides air conditioning and communications to the occupant. The suit consists of a main body, replaceable gloves and boots, and a detachable helmet.

The development of BISS included a materials test program. A flexible material was needed that could withstand the sterilization environment, that would be impervious to microorganisms, and that would be resistant to punctures and abrasions. After evaluating a series of materials, NASA selected a three-layer, laminated material consisting of one film layer of TFE Teflon™ fabric, one film layer of Viton™, and 0.125 mm (0.005 in.) bonded film layer of FEP Teflon™. The laminate has a total nominal thickness of 0.525 mm (0.021 in.). The FEP film can be heat-sealed to itself and serves as a leak-tight seal over the seams in the suit. One disadvantage of Teflon is its tendency to generate static electricity. A possible solution to this is to attach a thin conductive nylon cloth to the outside of the suit.

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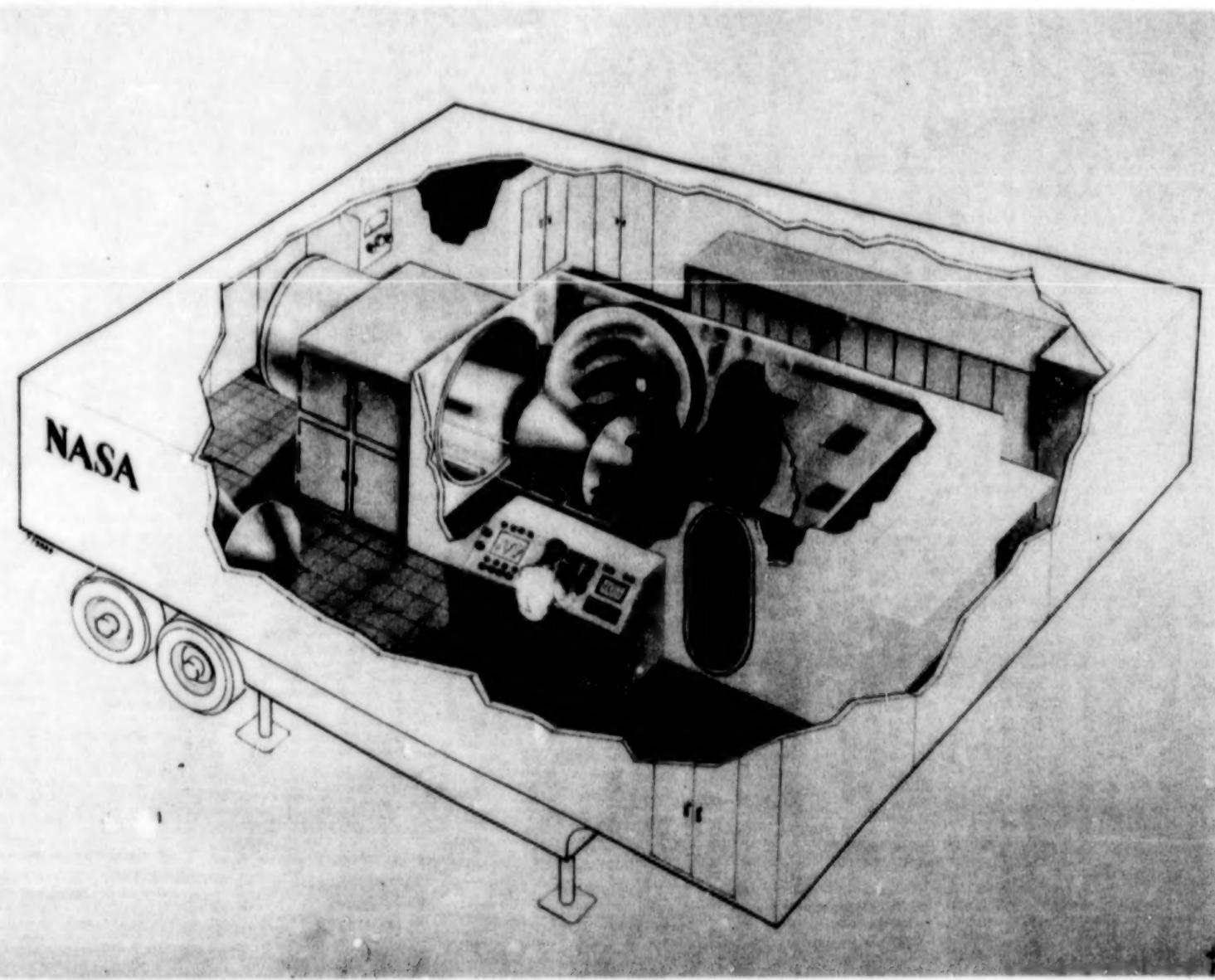


FIGURE 51. — Conceptual drawing of model assembly sterilizer.

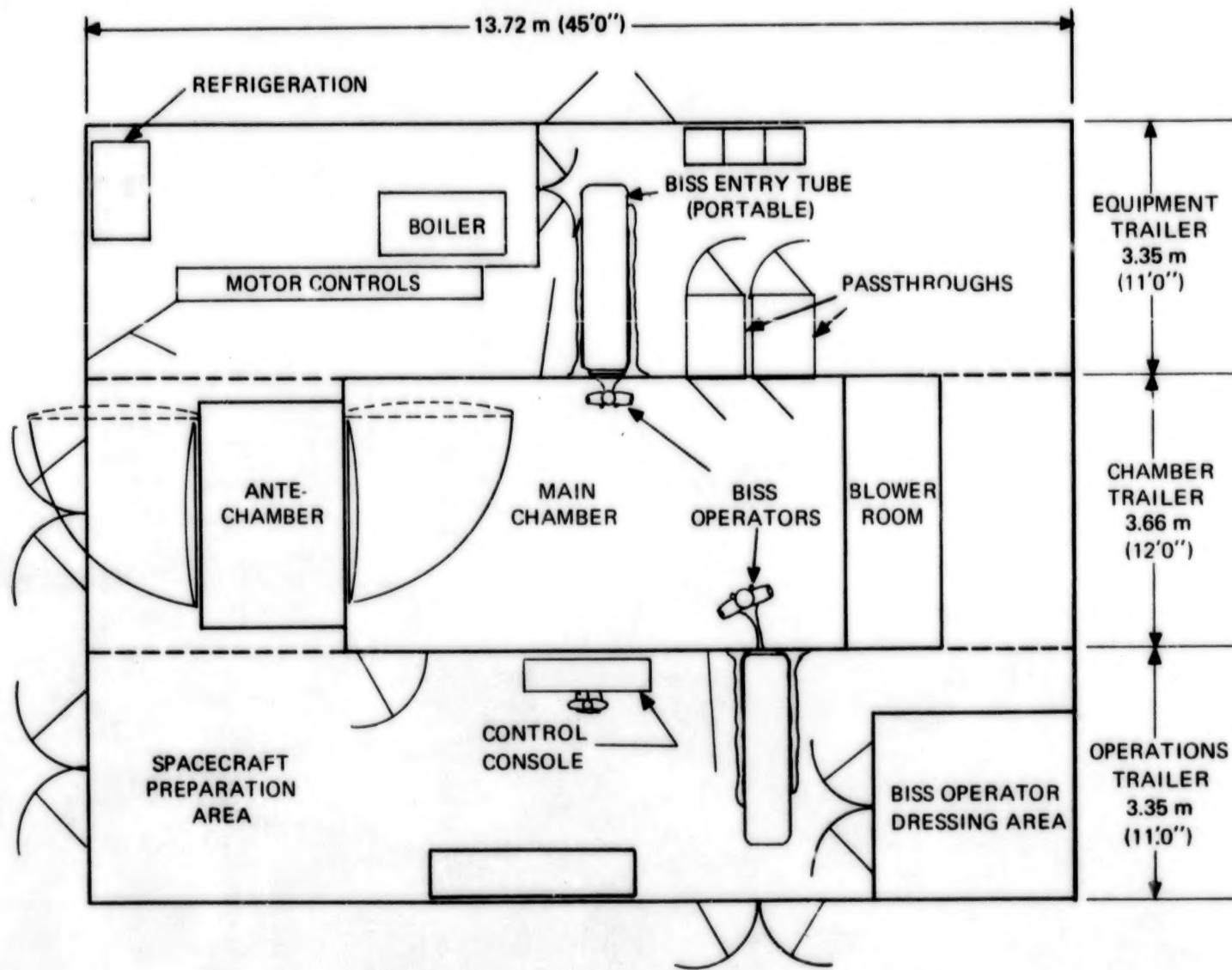


FIGURE 52. – Schematic diagram of model assembly sterilizer.

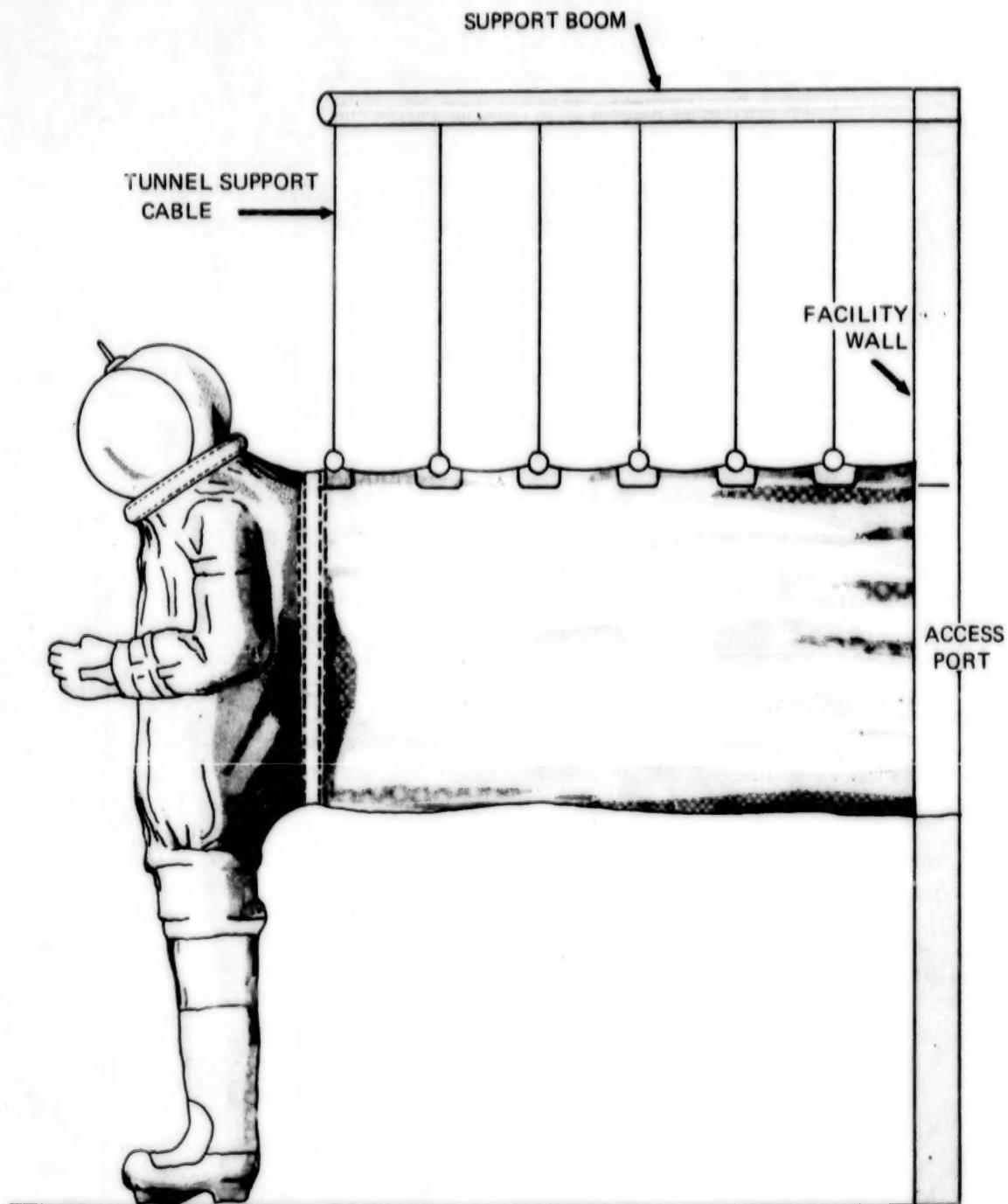


FIGURE 53. – Bio-isolator suit system (BISS) with tunnel and support mechanism.

Fabrication difficulties precluded use of the same suit material for the boots and gloves. A special rubber formulation was developed and this resulted in a suitable glove and boot that provided good dexterity and excellent puncture and abrasion resistance. The BISS helmet was fabricated from 0.15 cm (1/16 in.) aluminum with 0.31 cm (1/8 in.) thick Lexan™ faceplate.

Considerable study and design effort was required for the development of a donning mechanism to facilitate ingress and egress from the suit and tunnel. Suit-donning devices include boot holders to restrain the boots, a helmet hook to support the helmet in an upright position, and a hard tube for "reefing" the tunnel. Reefing is the process by which the suit tunnel is pulled over a hard tube to facilitate donning the suit. First, the hard tube is placed at the entrance of the BISS tunnel. The flexible BISS tunnel is then pulled back over the rigid tube until the suit is at the end of the tube. The operator then enters the suit through the tube.

An interesting aspect of the BISS development program was the emergency egress tests. If an operator becomes unconscious while in the suit, a means of rapid removal is required. The unconscious operator is lifted to an upright position by a second BISS operator, who uses a small hoist attached to the overhead boom that supports the BISS tunnel. When the victim is clear of the floor, he is guided back to the hard tube as technicians on the outside reef the suit tunnel in a normal manner. The stricken operator is then removed within 2.5 minutes. As an alternative, the sterility of the chamber can be sacrificed and the operator can be rescued through an emergency access door in MAST.

Human factors tests were performed to determine whether the suit would limit operations because of operator fatigue. During the tests, the suited operators performed functions such as heavy lifting, knee bends, pushups, ladder climbing, and assembly operations. No unusual stress was apparent during four hours of continuous work. This was considered a practicable goal; however, additional tests revealed that psychological factors should be considered when BISS operators are screened and selected. Some candidates found it impossible to perform adequately within BISS.

The final report, assessing the biological integrity and engineering reliability of the MAST system (ref. 191), concluded that this type of sterile access system

was capable of achieving a high level of biological and engineering reliability.

Men have worked within the MAST facility and have performed complex and delicate assembly operations without compromising the sterility of either the environment or the end product. The technology has been developed; it now awaits application in the nonaerospace field.

Sterile Repair and Insertion

NASA faced the problem of how to provide access to a sterilized spacecraft encapsulated in a bioshield in order to service, repair, or replace parts, without violating the sterility of the spacecraft. To solve this problem, a method was developed to permit the transfer of previously sterilized, heat-sensitive items through the bioshield (ref. 192). The key to the solution was the incorporation of one or more access ports, covered by a plastic film, into the design of the bioshield.

After terminal sterilization, the inside of the spacecraft bioshield and the inside of the access-port film are sterile, although the external surfaces are contaminated by the surrounding environment. Insertion is accomplished by use of a service bag or box. The simplest device is a flexible film cabinet or glove box. The inside of the service box is sterile. It contains the necessary sterile hardware, a replacement film for the access-port covering and the required tools for insertion of parts. The exterior of the box is contaminated.

First the box is heat-sealed to the film on the access port so that the contamination on the external surface of the access-port film and on the exterior of the service box is enclosed within the lens-shaped, plastic pouch formed in the heat-sealing operation. A cut around the pouch along the center line of the heat-sealed seam permits removal of the pouch to provide access to the interior of the spacecraft. Figure 54 is a schematic of the concept.

Sterile repairs, replacements, and insertions can then be made through the glove box. The replacement plastic barrier is then installed across the opening and heat-sealed in place. The service box is removed by cutting along the center line of the second seam, leaving a resealed entry port suitable for repetition of the operation if needed later.

Although development of the concept was easy, proving it was difficult. First, feasibility of the "split

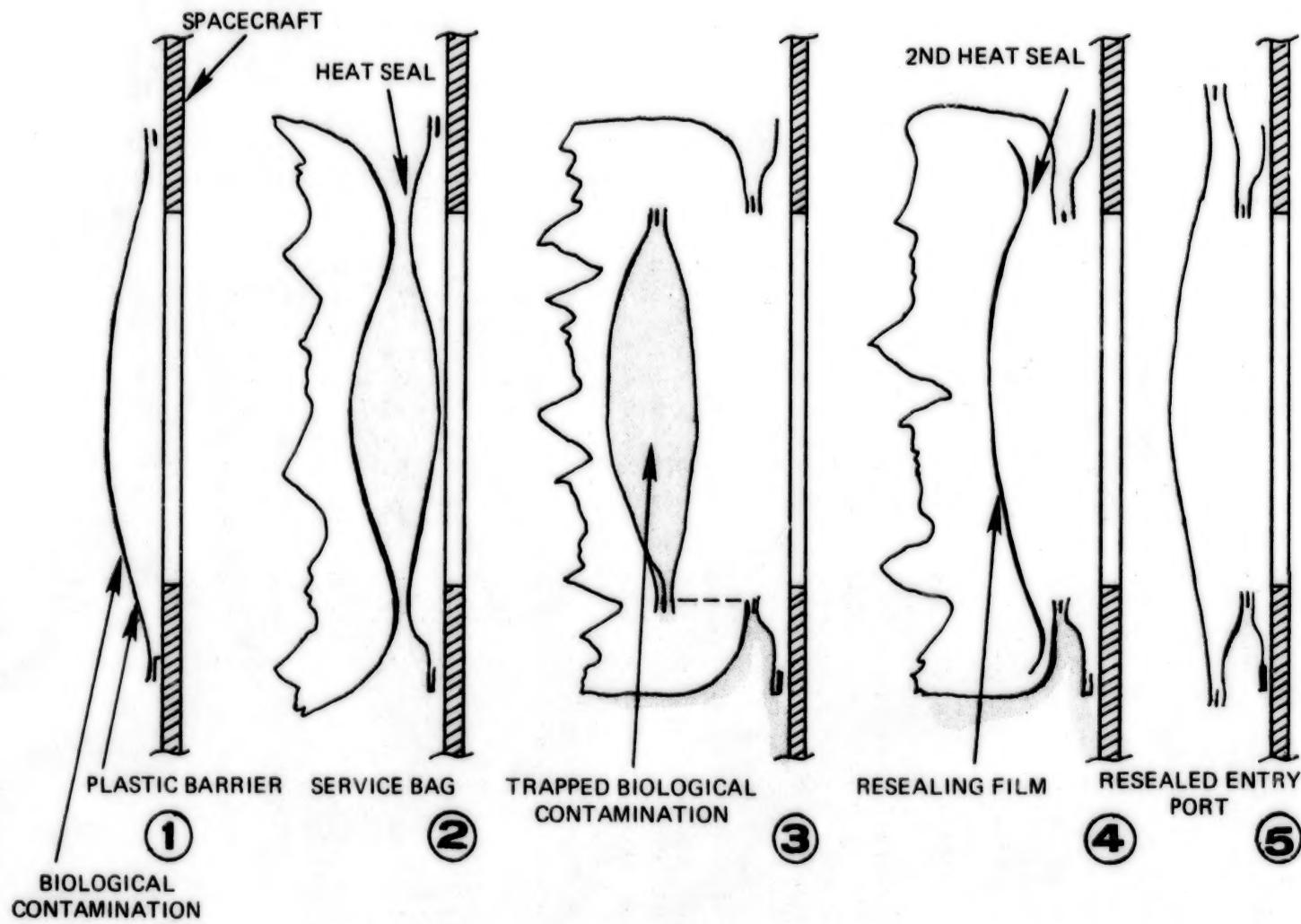


FIGURE 54. – General concept of sterile insertion.

"seam" technique (as the concept was called) was established. Hardware design concepts for self-propelled, operator-independent, heat-sealing, and seam-cutting tools had to be developed and evaluated. Concurrently, a materials evaluation program had to be performed.

A decision was made to use 0.125 mm (0.005 in.) and 0.250 mm (0.010 in.) fluorinated ethylene propylene (FEP) films as the plastic barrier materials, because of their good forming properties and high-temperature melting characteristics. The latter characteristics facilitated the sterilization of bacteria entrapped in the seam during the heat-sealing operation.

The cutting operation was recognized as the most critical step in the sterile insertion sequence. Before the program progressed further, evidence was needed to establish whether the molten plastic encapsulated the organisms sufficiently to preclude release of organisms during the cutting operation. Under suitable temperature, time, and pressure conditions for heat sealing, a homogeneous bond is formed that cannot be separated to permit recovery of entrapped organisms. This problem was resolved by using a nonheat-sealable insert of KaptonTM to permit separation after the heat-sealing operation. The experimental arrangement is shown in figure 55. Homogeneous seams impregnated with bacterial spores were also prepared without inserts and were evaluated for the release of microorganisms during the cutting operation.

The resulting data indicated acceptable reliability for the cutting operation. Better than 99.9 percent of the organisms present were killed during the heat-sealing operation. Based on these results, the development of prototype sterile insertion equipment was initiated (ref. 193). These efforts included the design and fabrication of self-propelled, heat-sealing and cutting tools. Figure 56 depicts the self-propelled, heat-sealing tool in operation. The test program included the evaluation of materials and seam before and after sterilization, and operational demonstrations of the complete system. In addition, new molding technology evolved in the process of fabricating the large plastic barriers.

In summary, these programs were successful in all respects. The prototype units have a reasonable range of performance capability and are completely compatible with sterilization processes. Seams made under simulated operational conditions showed no

imperfections that would impair biological security. After cutting, seam edges exhibited homogeneous seal characteristics. Overall, these studies led to the conclusion that a sterile encapsulated spacecraft could be entered and serviced without violating its sterility.

NONAEROSPACE APPLICATIONS

A wealth of technological advancements have resulted from NASA's investigations into sterile assembly techniques. Nonaerospace applications of these developments are limited only by one's ability to recognize how the technology can be adapted to particular needs.

Medicine

The maintenance of a sterile environment is essential to various fields of medicine. For example, sterile assembly techniques could improve:

- Patient isolation, particularly for cases of highly contagious diseases
- Surgical procedures, by using the sterile environments as safeguards against bacterial infection of exposed internal organs and tissues. (This sterility may be particularly important for operations on patients with low resistance.)
- Postoperative care, when prevention of infection is of paramount importance.

Despite awareness of these applications, isolator costs and manipulative constraints have limited their use in the past. The advent of low-cost, plastic isolators equipped with surgical gloves removed these constraints. Accelerated research can now be expected in the application of isolator technology to the medical field. In fact, research studies on isolators are presently being conducted in hospitals around the country.

A particularly important use of isolator technology has been developed for burned patients. Since the body's defense mechanisms cannot resist secondary infections that result from third-degree burns, the patient usually dies. However, placing burned patients in a germ-free, plastic isolator achieves effective protection against hospital pathogens. In this way, an appreciable recovery of a patient with third-degree burns can be obtained.

MAST also has many medical applications. MAST is used in conjunction with federal agencies for a

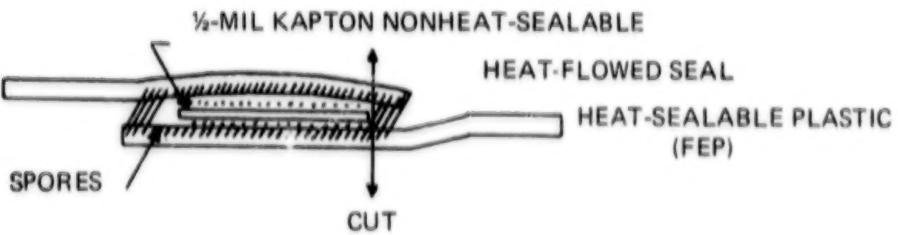
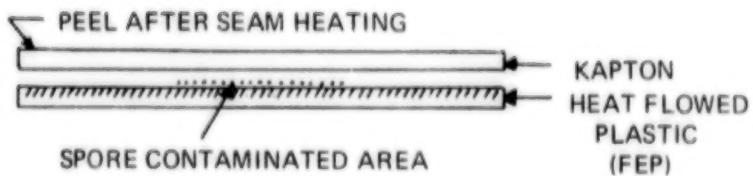
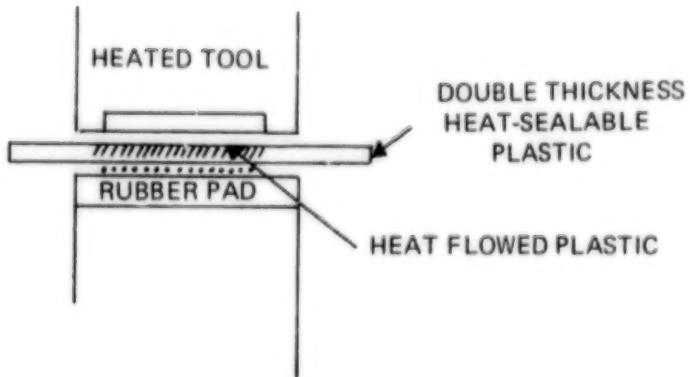
METHOD 1. NONHEAT-SEALABLE INSERT**METHOD 2. DISSIMILAR FILMS****METHOD 3. SINGLE CONTAMINATED FILM**

FIGURE 55. — Separation techniques.

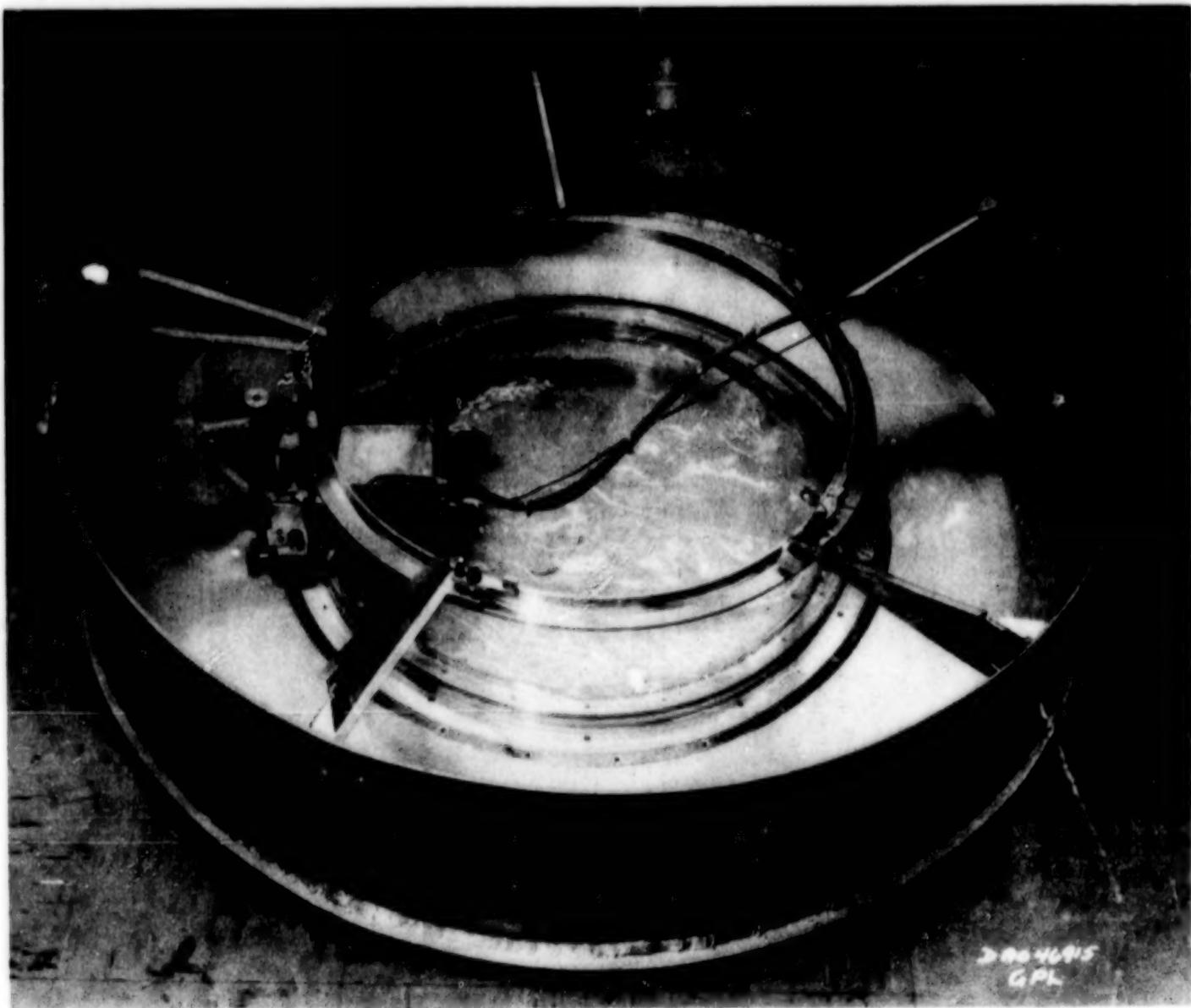


FIGURE 56. - Heat sealing tool clamped to the port opening rail inside the sterile repair test apparatus.

variety of research projects including preventive medicine, burn patient care, environmental toxicology, and cancer research. Both types of biological barriers are used—in burn patient care, the subject is protected from the external environment, and in cancer research studies MAST protects the operators from extremely hazardous tumor viruses. It has been estimated that the use of MAST will gain two years' time in the fight against viruses that cause tumors.

Pharmaceutical Manufacturing

Maintaining sterility of pharmaceutical products is a continuing major concern of the industry, particularly when biological processes are used in the manufacturing operations (such as in the production of antibiotics and serums). In addition, quality assurance by the manufacturers and government-monitoring agencies entail analysis of samples of very expensive products. Inadvertent contamination during sampling can result in significant losses to the manufacturer. Fortunately, the sterile assembly techniques evolved by NASA can ease these problems. For example, product sampling under glove-box conditions can minimize the contaminated-sample problem.

Cosmetic Manufacturing

The cosmetic industry is also plagued with contamination problems similar to, but not as pronounced as, those in the pharmaceutical industry. One use of the sterile assembly technique is being evaluated for the manufacture of talc, the basic constituent of most body and cosmetic powders. In the past, bacterial contaminants have caused adverse dermatological reactions. These have sometimes led to the removal of products from the market place. Adaptation of ETO into the processing operation was investigated as a method of eliminating this problem. It was found that, once sterilized, the products could be packaged under aseptic conditions.

Food and Industrial Packaging

The molding technology developed for FEP film can be adapted to produce special packaging which requires inertness, environmental stability, and immunity to higher-than-normal temperatures. Modifica-

tions to the mobile, heat-sealing unit can be made so that it remains stationary. In this way, plastic-wrapped items can be sealed by running them through the sealer.

HORIZONS

While the use of biological barriers is at least as old as man's first attempts to prevent spoilage in his food supplies and to isolate persons with communicable diseases; the parameters of interest and concern for the control of biological material have perhaps expanded more in the past two decades than in all previous, recorded time.

The diverse areas of use for biological barrier techniques include the:

- maintenance of other planets as ecological preserves; e.g., spacecraft sterilization;
- prevention of possible back contamination from other planets;
- protection of hospital patients during and after operative procedures;
- isolation of burned patients and patients who are uniquely susceptible to environmental microbes;
- protection of research workers handling infectious disease agents;
- protection of researchers handling oncogenic viruses during studies on the etiology of leukemia and other cancers;
- safe production, packaging, and distribution of drugs and pharmaceuticals;
- controlled handling of animals used in research in order to prevent the spread of zoonotic disease; and
- safe shipment and transportation of infectious microbial cultures and medical specimens.

Looking at broader problem areas, one begins to realize that the degradation of our natural water resources and the ever present and increasing problem of air pollution are partially problems of contamination control that could use the principles of biological barriers. In fact, as our society becomes more complex, it seems clear that the need for various types of biological barriers is increasing and that the level at which contamination control applies become more and more microscopic.

With regard to the specific developments in biological barrier technology that have taken place in

the past decade, no one would be so bold as to suggest that a panacea for all current problems is at hand. Nevertheless, the research ingenuity and finesse being applied by NASA and the medical and biological professions to solve contamination control problems has resulted in a literal revolution in methodology. Moreover, an amazing amount of cross-fertilization has occurred. For example, the techniques of the pharmaceutical industry have been helpful in studying the problems of spacecraft sterilization, while the procedures for rearing germ-free animals are applicable to certain hospital situations. Perhaps the single most recognized development has been the application of aerodynamic techniques in providing minimum turbulence air flow streams (laminar flow) for operations requiring contamination control.

THE LAMINAR DOWNFLOW CLEAN ROOM

The laminar flow clean room, unlike many other biological barriers, has self-cleaning capabilities. Airborne particles in the clean room are carried out of the room by a downward flow of air. (The term "airborne particles" as used here, refers to all particles in the airborne condition regardless of material or origin, such as biological cells, dust or dirt particles, liquid droplets, etc.) The laminar downflow clean room provides the additional advantage of a dual barrier. It excludes external contamination from the room, and internal room barriers prevent cross-contamination within the clean room.

PRINCIPLES OF LAMINAR AIRFLOW

The principal function of a clean room is to control airborne particles by preventing them from entering an area or enclosure. This is accomplished by passing all the air through a highly efficient filter system. Ability to maintain a high level of air cleanliness by filtration is dependent on the airflow characteristics and the activities performed within the enclosure. Providing a unidirectional airflow through the enclosure is an effective means of removing generated airborne particles and preventing a buildup of contaminants in the laminar airflow device.

Laminar airflow is defined as "airflow in which the entire body of air within a confined area moves with uniform velocity along parallel flow lines" (ref. 194). A laminar airflow clean room or device is a dynamic

facility in which the attainable cleanliness level depends on the performance of its basic control elements rather than the procedural and maintenance controls employed in nonlaminar flow facilities.

Basic Elements

The basic requirements for a laminar airflow clean room are:

- The airflow must be uniform in velocity and direction throughout any given cross section of the clean room (ref. 195).
- All air entering the enclosure must pass through the high efficiency particulate air (HEPA) filter system (with no leakage in or around the filter media) (ref. 195).

A clean room operating to meet these basic requirements will maintain the air throughout the room at the same level of cleanliness at which it flows from the filter system—provided there is nothing already in or introduced into the enclosure that disrupts the airflow, or that introduces contaminants into the air-stream. The HEPA filter is capable of capturing a minimum of 99.97 percent of all particles 0.3×10^{-6} m (0.3 micron) and larger. This provides air consisting of a relatively low number of significantly sized particles.

Air cleanliness levels or classes are based on actual particle count. The levels are defined by the maximum number of particles of a given size, allowable per unit volume. Classes of air cleanliness are further defined in Fed. Std. 209b, paragraph 5 (ref. 194). To obtain a reasonably true indication of the class or level of cleanliness, particle counts should be taken during normal operational activities, and at or near critical or pertinent locations where work is being performed. The basic concept of vertical laminar airflow (VLF) is shown in figure 57. The direction of airflow is from top to bottom. In this type of facility the air flows into the enclosure through the ceiling, which is composed of HEPA filters, and is exhausted from the enclosure through the floor grating.

Other Elements of Control

Other conditions that may be controlled by a clean room or device are:

Other Types of Contaminants.—These are principally the gases that are generated within the en-

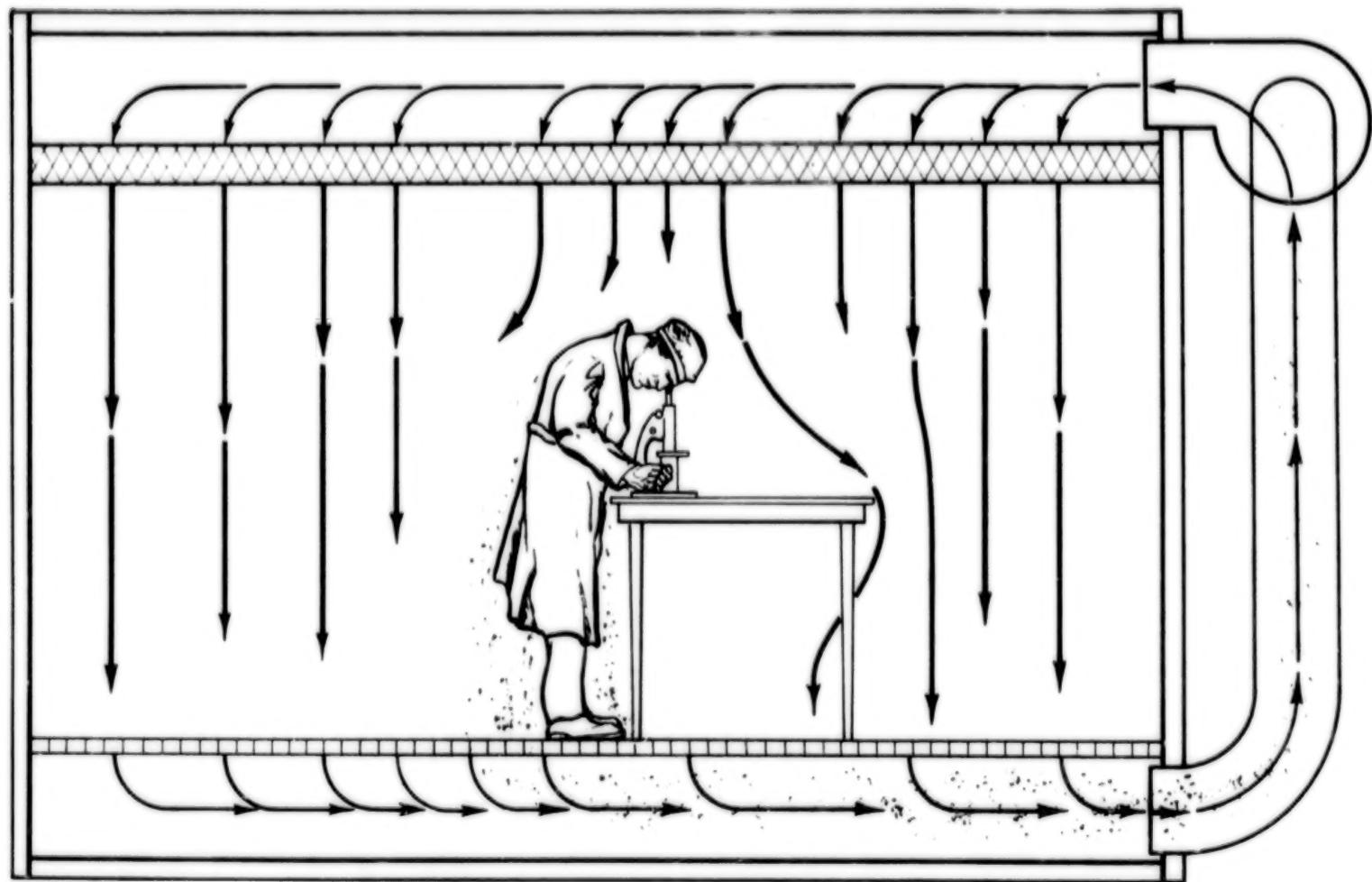


FIGURE 57. — Laminar flow clean room.

closure, or are introduced into the air-supply system and are not removed by the HEPA filter. Controls may be incorporated in the air supply system as required for personnel safety or reduced product effects.

Temperature.—This condition is normally controlled for personnel comfort, but also may be specifically controlled to meet product requirements.

Humidity.—This is also a condition controlled for personnel comfort; but more specifically, humidity is regulated to control its effects on products and tools and to limit problems caused by static electricity.

Functional Characteristics

The functional characteristics of the laminar airflow principle employed in the clean rooms and devices may be summarized as follows:

Clean-Down Capability.—The air velocities are sufficient to remove airborne contamination generated within or carried into the facility. At an air velocity of 27.4 m (90 ft) per minute, a vertical, laminar airflow room with a 3.05-meter (10-foot) ceiling would undergo a complete change of air in less than 7 seconds.

Unidirectional Airflow Pattern.—Any particle capable of being airborne will be carried away from the product by the airstream because of the single direction of the airflow pattern. This minimizes the opportunity for airborne particles to contaminate the product (ref. 196).

Reduced Janitorial Maintenance.—The ability of the facility to exhaust the airborne contamination produced within it precludes the need to continuously vacuum the area to maintain the desired air cleanliness levels.

Class 100 Available.—All laminar downflow clean rooms are fully capable of attaining a Class 100 condition (some types only in part, depending on the operations). This class or better can be maintained with reliable personnel and proper control of operations.

Better Control of Humidity and Temperature.—The volume of air introduced into the area is so much greater than in other types of environmentally controlled equipment that the absorption of heat, cold, moisture, or dryness is distributed over a greater volume of air. This greatly simplifies the maintenance of the desired levels. The recirculated portion of the air is also less costly to recondition because it has deviated from the norm much less than if it remained in the room for a longer period.

Reduced Garmenting Restrictions.—In a majority of circumstances, garmenting requirements may be met by a smock and head covering. This is largely due to the capability of the laminar airflow to carry away particulate matter emitted by the worker. This represents a sizable savings in manhours. It eliminates the time consuming robing and disrobing several times each day which is required when all-encompassing garments are worn. The additional freedom of movement frequently manifests itself in improved output as well.

Reduced Critical Need for Airlocks, Air Showers, Etc.—Except in unusual cases (microbial control requirements or sterile conditions), air showers, double-door airlocks, and large dressing rooms are not usually considered for use in laminar airflow installation.

Increased Volume of Air Required.—The volume and speed of air requires air moving equipment and ductwork of larger size and capability than used for other types of facilities.

THE DOWNGLOW CLEAN ROOM

In a downflow room, the air flows vertically downward from the ceiling (made up of HEPA filters) and is exhausted through a grated floor. A section of a downflow room is shown in figure 58.

The downflow room normally provides uniform control of airborne particulate matter throughout the entire clean room area. A properly designed downflow clean room provides the following advantages over other types of rooms:

- It operates well within the Class 100 level.
- It operates effectively over a wide range of air velocities.
- It ensures rapid removal of generated or introduced contaminants from the room.
- It reduces cross-contamination between adjacent operations.
- It reduces janitorial and maintenance costs.

Basic Design Requirements

Some of the basic design requirements that have a significant effect on the operation of a downflow room are listed below:

Ceiling.—The supporting framework must be structurally adequate to support the HEPA filters, light fixtures, sprinklers, and other ceiling fixtures. It is imperative that a positive air seal be maintained

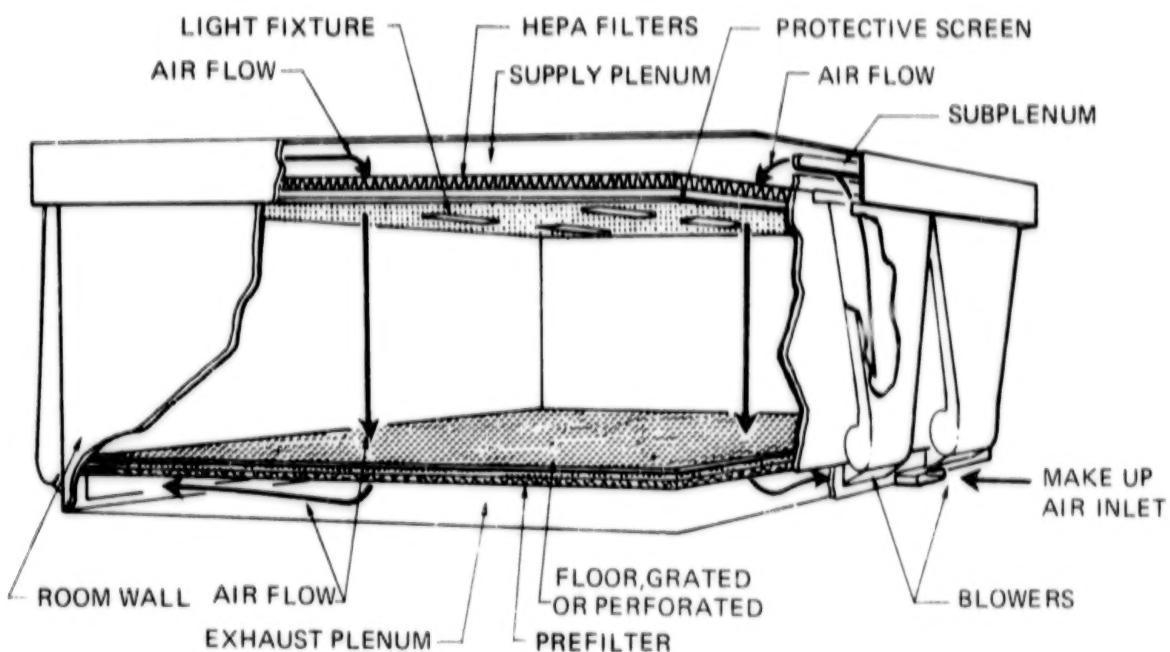


FIGURE 58. – Elements of a downflow room.

between the support frame and HEPA filter frame. There must be no air leaks in the framework itself or around fixtures such as lights, sprinkler heads, etc. Figure 59 shows some possible sources of air leaks in a filter bank installation. A protective grille should always be provided on the room side of the HEPA filter bank to prevent damage to the filter media.

The filter support frame should be designed to allow only a minimum area between filter units. The support frame reduces the effective filter area and creates air turbulences downstream from the filter. Figure 60 illustrates the air turbulence created by the support frame between filters. The major turbulent area, in which the air circulates back to the top, extends downstream a distance about three times the width of the support frame where there is a unidirectional flow of air on both sides. Downstream from this area, minor air disturbances may extend all the way to the floor, gradually diminishing in degree. These disturbances are moving at the same speed and direction as the normal airflow pattern; therefore, it is believed that they present no major problem—except possibly for the most critical type of operation. The major turbulent areas are limited in the distance to which they extend. They do not present any serious problem, except when the work areas

are close to the ceiling or the width and subsequent depth of the area are extensive.

Mounted Ceiling Lights.—Light fixtures mounted on or in the ceiling present the same problems of air turbulence as the ceiling support frame. The total width of the fixture should be minimized to shorten the downstream turbulence area. Fixtures should be mounted between filters as shown in figure 61, or mounted on the support frame as shown in figure 62. The suspended fixture illustrated in figure 61 extends the turbulent area further into the room, and this can be an undesirable feature. Single tube fixtures with a width about equal to the width of the support frame and mounted directly to the frame, create minimal air turbulence. If the turbulent areas do not extend into a dirty area or work area in the room, they should not present any major problem for normal room operation. Determining the best type of light fixture and the best method of mounting is dependent on: the type of support frame and filter mounting assembly used; the available access for servicing lights; the light distribution and intensity required; and the ceiling height.

Walls.—The interior surface of the walls should have a smooth, hard-gloss finish. Strips, ledges, and offsets should be kept to a minimum. Doors and

windows must be flush with the interior surface. Any uneven wall surface will create a small amount of air drag; therefore, the inner wall surface and the effective edge of the HEPA filter should be as close together as possible at the wall and ceiling juncture. A wall offset at this point will create a major air turbulence that will extend downstream along a wall surface such as a ledge, shelf, lamp fixture, etc. The downstream turbulence will extend from the bottom side of the projection for the same distance-to-width ratio shown in figure 63.

Floor.—The entire floor area serves as an air exhaust. The floor should be constructed of grating or perforated metal which should have a minimal 60-percent opening for air passage. The floor should be designed to permit adjustment of the air passage opening to facilitate airflow balance within the room. Methods used to adjust the floor openings include varying the hole sizes in the grating or perforated sheet, varying the thickness of the prefilter media

(directly under the floor), and using adjustable dampers or baffles (which facilitate a more uniform airflow) located directly beneath the grated floor. The floor should be laid out in sections with adequate structural support to meet floor loading requirements. Sections should be removable and should be of a size and weight that can be safely handled by two men. The floor material should meet the operational requirements of the clean room. In most instances, either plated steel or aluminum is satisfactory; however, some operations may require stainless steel.

Prefilters.—In the VLF room, the prefilter is usually located just under the floor. A sheet or pad media is normally used and is held in place by a coarse mesh screen. The filter media catches small items that fall through the floor openings. It also creates some air resistance to aid the airflow balance in the room. Located just under the floor, the media can be cleaned and replaced by removing floor sections. Locating the prefilter at other points in the

- NOTES:
1. LEAKS IN FILTER MEDIA.
 2. LEAKS IN BOND BETWEEN MEDIA AND FRAME.
 3. LEAK IN GASKET BETWEEN FILTER FRAME AND SUPPORT FRAME.
 4. LEAK IN SUPPORT FRAME.
 5. LEAK BETWEEN SUPPORT FRAME AND WALL.

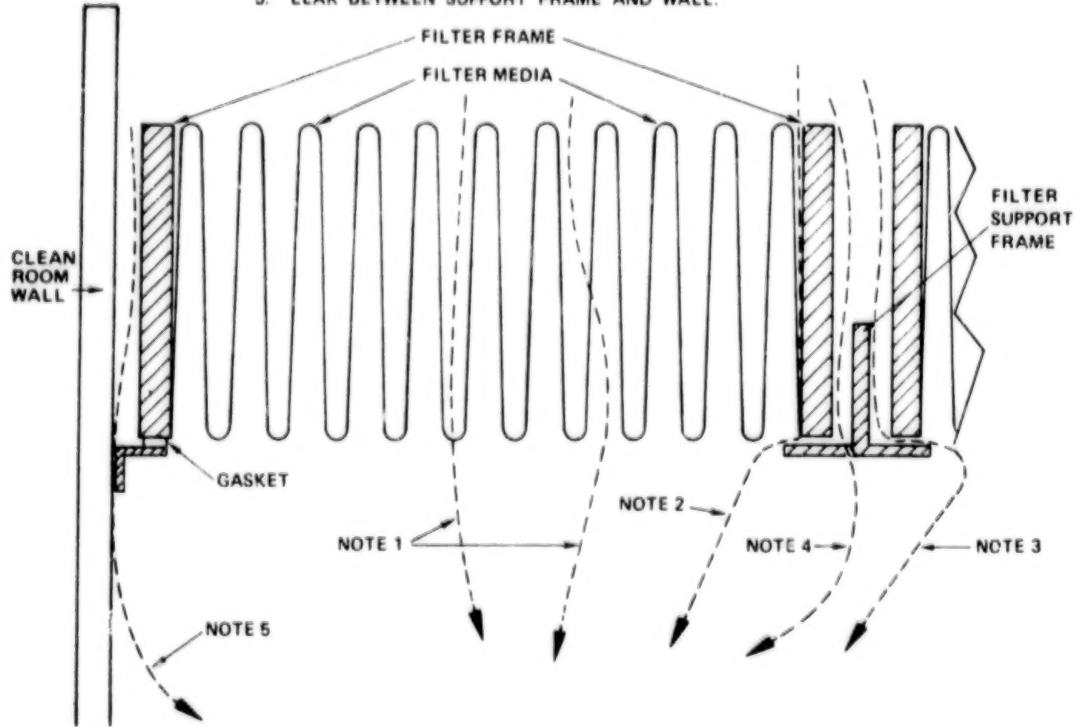


FIGURE 59. — Filter leaks.

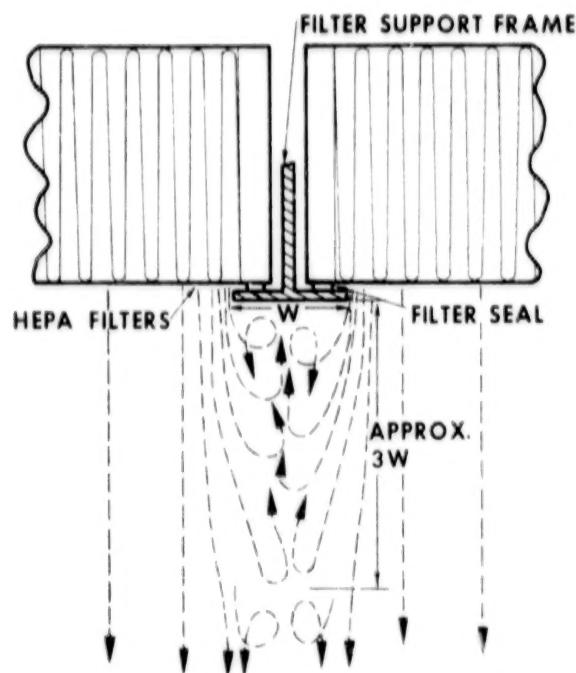


FIGURE 60. — Airflow turbulence.

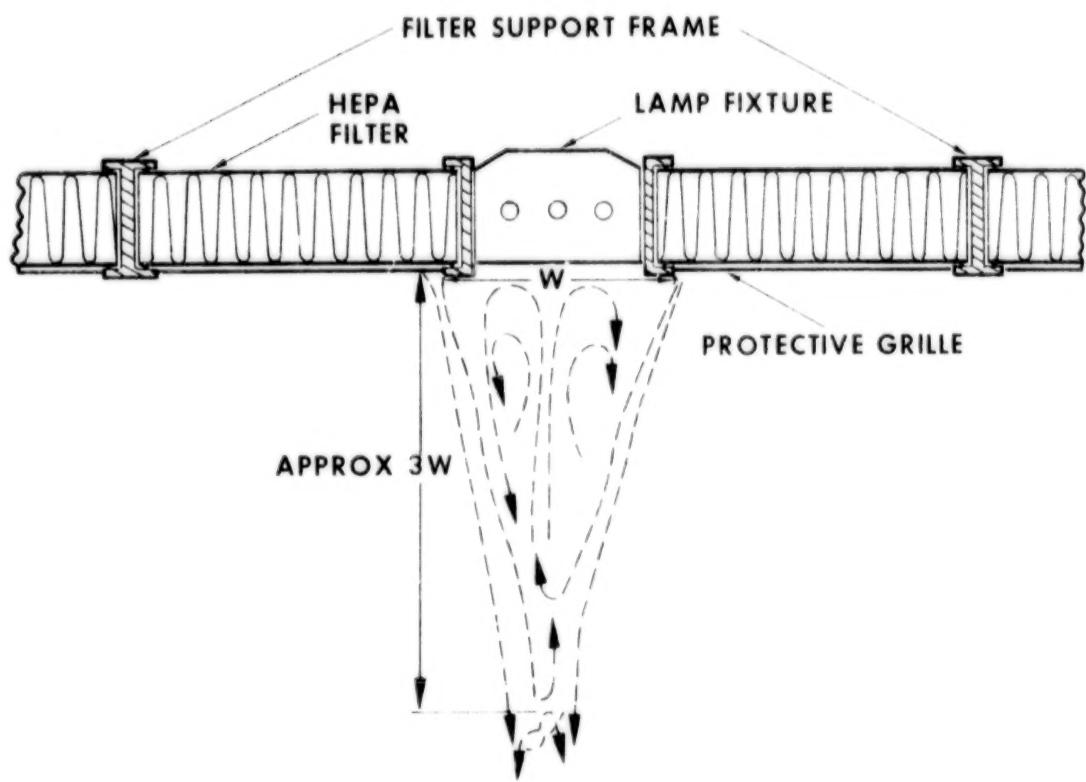


FIGURE 61. — Airflow turbulence below lamp.

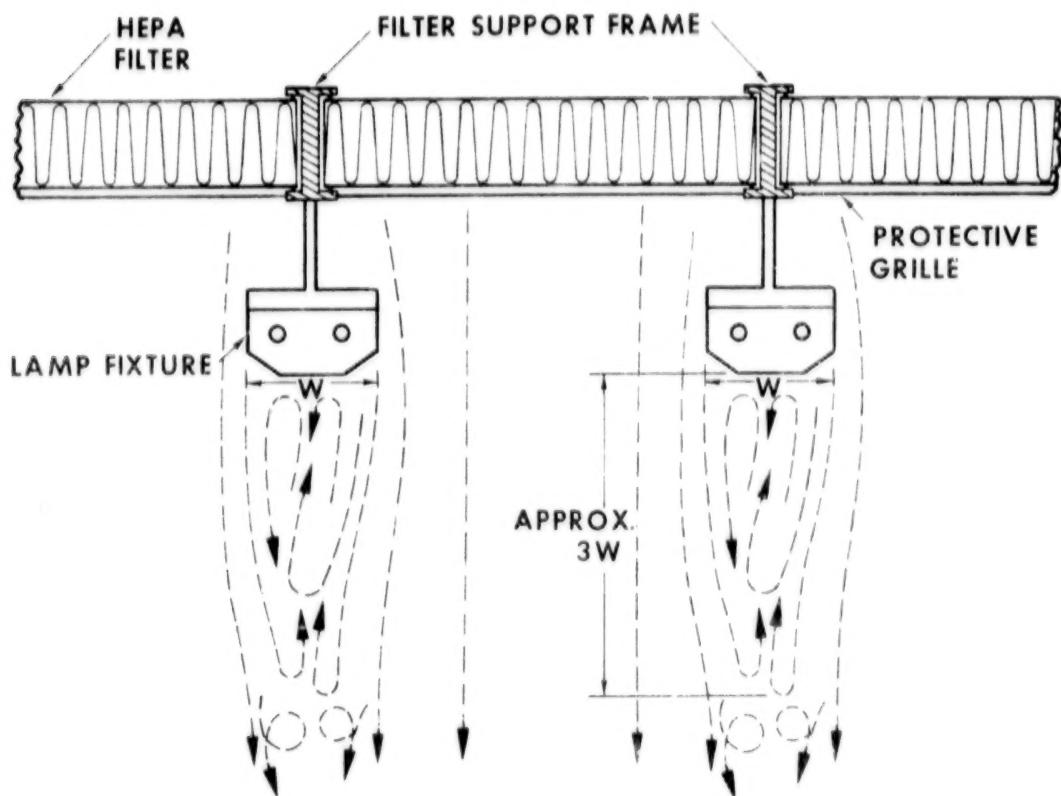


FIGURE 62. – Airflow turbulence, suspended lamp.

system may be satisfactory when access to the prefilters is practical for cleaning or replacement and when the type of filter media used in the space provided is adequate to accommodate the airflow volume.

Plenums and Ducts.—The downflow room employs an air recirculating system that is composed of an exhaust plenum under the floor and return air ducts that connect to the supply plenum. This system must be of sufficient size to handle the airflow at the required rates without noise or vibration. The air-handling system must include facilities for introducing makeup air and for tempering the air to the desired temperature and humidity.

Pressure Differential.—A positive pressure differential between the room and adjacent areas should be a 0.05-inch water gage minimum with all entryways closed. With entryways open, the airflow capacity should be adequate to maintain an outward flow of air. Inability to maintain minimum overpressure in the room is an indication of inadequate air-moving equipment, or excessive leaks in the room or air-handling system.

LAMINAR DOWNFLOW CURTAIN ROOM (PORTABLE)

The portable curtain room is an adaptation of the downflow room. The portable room differs in three ways: (1) the air is exhausted under the walls of the unit all around the perimeter, (2) the air is not recirculated, and (3) the side walls are plastic curtains. The portable room is capable of being transported, and it can envelop heavy, cumbersome components or structures less capable of being transported to a standard downflow clean room. A typical downflow curtain room is illustrated in figure 64. This room is capable of operating within the Class 100 level and can be a completely self-contained unit except for electric power.

Design Requirements

Design requirements are the same as for the VLF room except as stated in the following paragraphs.

Walls.—The walls are plastic curtains which should be attached to the ceiling filter bank in a way that

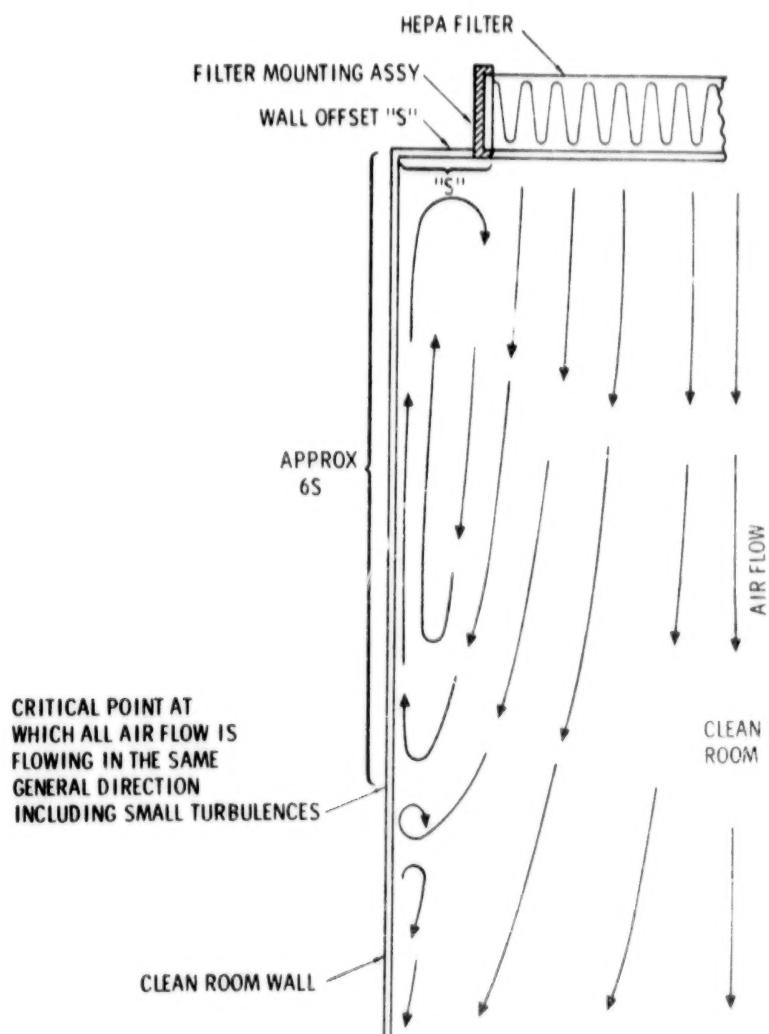


FIGURE 63. — Airflow turbulence from wall offset.

precludes any air leaks in this area. The inside surface of the curtains touch the effective edge of the filter to eliminate any offset and resulting air turbulence as shown in figure 63.

The curtains should preferably be a nonstatic, plastic material and should extend to within 30.5 cm (12 inches) of the floor. Weighting along the bottom edge may be required to assure an unwrinkled, even surface for uniform airflow. Seams in the material should be vertical. Any access junctures should have zipper closures that are as airtight as possible and should have a smooth surface on the interior side of the curtain.

Floor.—The portable room has no floor of its own and may be set up on any surface that will support

it, the product, and the activities performed in the room. Care should be exercised to prevent a rise of dirt from a dirty floor. The effective clean work area should be at least 45.7 cm (18 inches) above the bottom of the curtain walls.

Lighting.—Mounted ceiling light fixtures should receive the same considerations as for the downflow room. Overhead lighting may also be augmented by light through the transparent curtain walls as required.

Prefilters.—In the portable room, the prefilter is usually located at the inlet to the air blower system. Because the unit may be operated in exceptionally dirty areas, the prefilter requires frequent inspection, cleaning, and replacement to prevent overloading.

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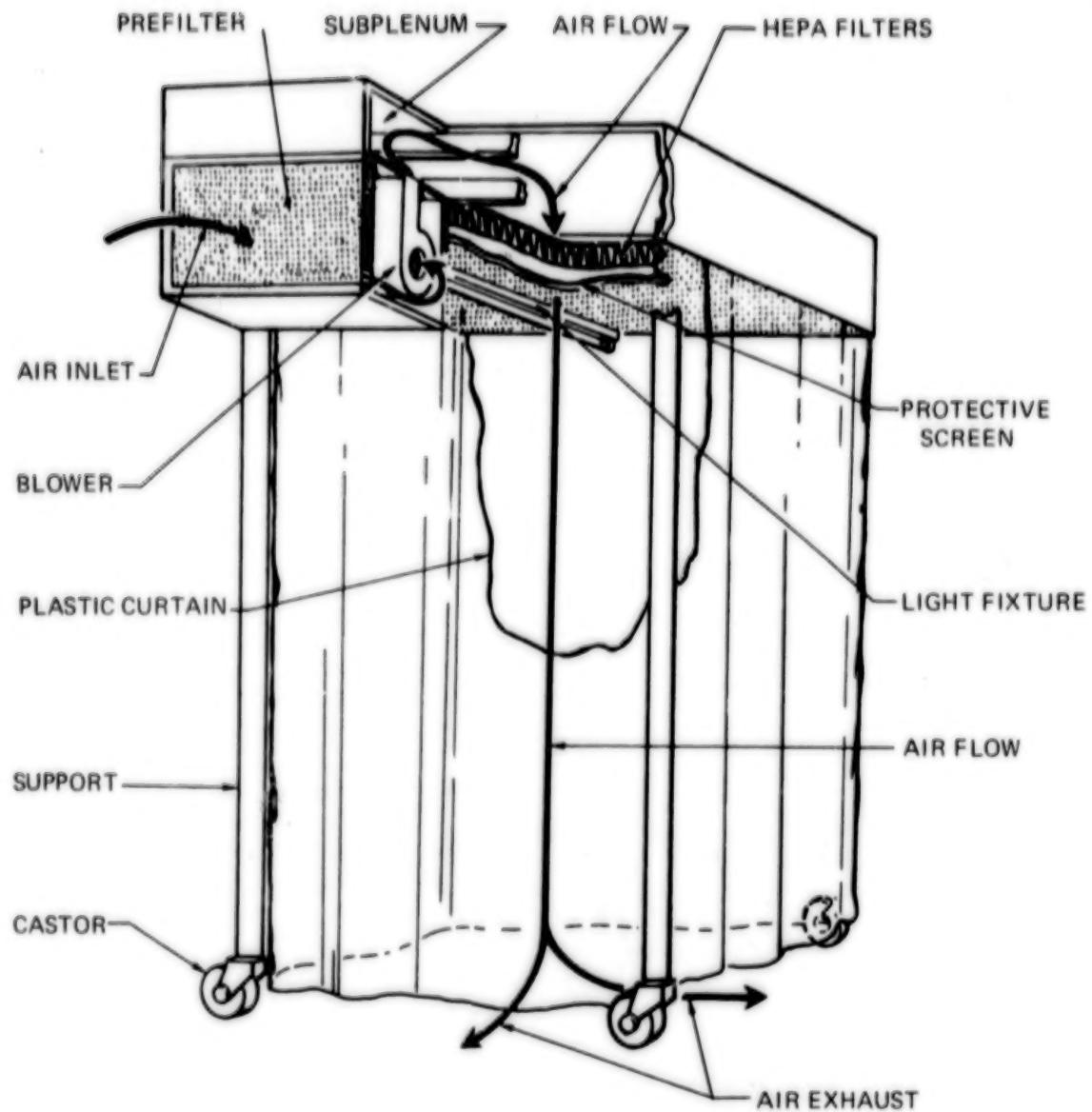


FIGURE 64. – Downflow curtain unit.

Air Velocity.—The normal velocity of 27.4 m/min (± 3.05 m/min (90 ft/min ± 10 ft/min) will maintain a Class 100 condition; however, severe conditions may necessitate higher airflow velocities.

Pressure Differential.—With the open perimeter exhaust system, no pressure differential is maintained.

Supports.—Legs supporting the curtained downflow room should be rigid with height restrictions commensurate with required balance. Casters may be used with the legs to provide lateral movement. Lift rings may be provided on the top of the unit to facilitate lifting and movement (by a crane), or for suspending the unit over the work area.

USING THE DOWNGLOW CLEAN ROOM

A properly operating downflow clean room provides near sterile air in the room (Class 100 or better), excellent isolation of personnel from the critical work, and the rapid removal of airborne particles from the room. Good clean room operating conditions demand that the critical work be positioned in the upstream of the airflow—or at least across the airstream, horizontal to the worker and other equipment. This is extremely important, because undisturbed downward airflow can then funnel off contaminating particles before they reach the critical work site.

Layout

The downflow clean room is specifically laid out so that the downflow of air can be an effective barrier to contamination. All equipment located in the clean room should be installed to:

- permit the clean air to flow around all sides of the equipment;
- expose sensitive work areas to unobstructed airflow;
- minimize the size of the horizontal cross sections of equipment to reduce airflow turbulence;
- provide adequate space for clean room workers to perform required tasks downstream, or at least to one side of the sensitive work; and
- provide adequate space for movement of personnel and material in the clean room, and to avoid disturbance of airflow near the sensitive areas.

Personnel Clothing

Personnel clothing must be provided to prevent worker contamination from reaching the sensitive product. Clothing requirements vary greatly, depending on the degree of protection required. If the product is to be kept sterile while exposed in the clean room; the worker must wear a close fitting hood, a smock with close fitting wrist cuffs and good neck closure, and gloves. A "bunny suit" or coverall garment may be preferable to the smock for some applications. For less stringent requirements, only gloves may be needed to prevent direct transfer of contamination to the product. For moderate requirements, head covering, gloves, and smock may be adequate protection.

Good personnel practices must be developed by training clean room workers to understand how contamination reaches the product, (figure 65) and the necessity of meeting cleanliness requirements.

Monitoring (ref. 197).

The clean room worker should be taught the modes of contamination transfer and the sources of contaminants for a given area. Personnel and equipment are the two main sources of contamination, either generated within or carried into the clean room. Contamination from personnel is in the form of skin flakes, skin oils, dandruff, clothing fibers, and outside dirt carried into the clean room on clothing. Equipment contamination is caused by particles loosened from wear, vacuum pump oil and lubricant droplets, corrosion of metals, residual dirt from equipment improperly cleaned, inadequately controlled chemical reactions, unvented cleaning stations, etc.

Work Location Monitoring

Work location monitoring is the measurement of airborne particle concentrations in a defined zone—usually within a large area such as a clean room or clean bench. Work location monitoring can be applied to any specific area; however, it is probably most useful during critical operations requiring extreme particulate control. This is especially true in highly controlled areas where the only measurable airborne particle levels occur as a result of a procedure involving the critical work piece of component.

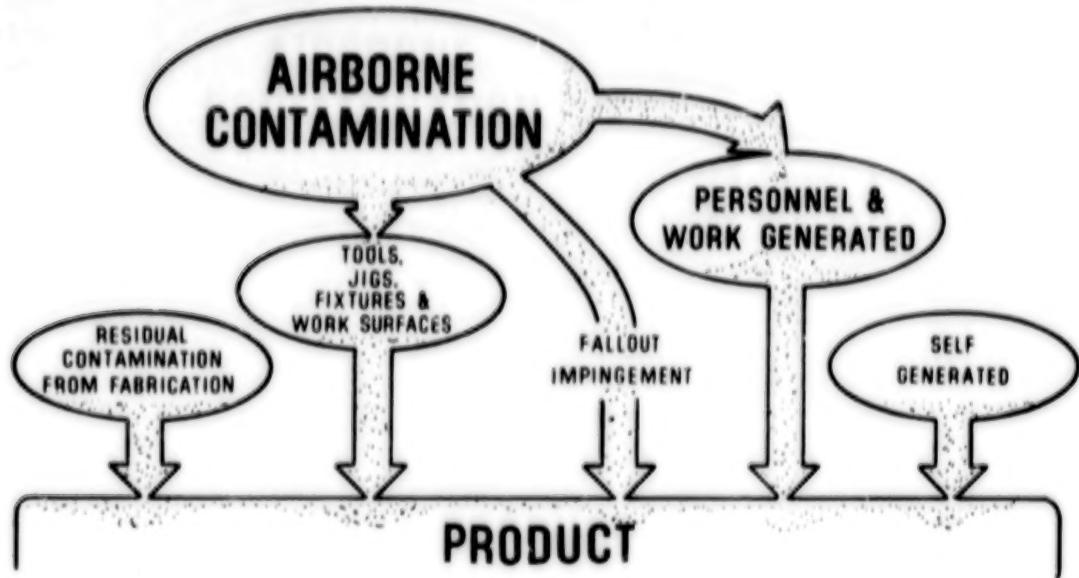


FIGURE 65. - Contamination paths to product.

In such cases, the airborne particle contamination is highly localized in one area and can be measured only in that area.

The work location may be monitored during an initial setup or after any significant change in a work location operation. Subsequent monitoring may be done on a periodic spot-check or continuous basis to assure the user that airborne particle concentrations are within desired levels. The frequency of monitoring can usually be decreased in a highly repetitive or production line situation in which procedural changes are almost nonexistent.

Because of the expense and work involved, continuous work location monitoring should only be considered for highly critical operations. Continuous monitoring provides the advantage of being sensitive to even the slightest procedural changes and immediately indicates when particle concentrations exceed a desired control level.

It must be remembered that some fluctuation in particle concentration will occur during most operations. At best, particle counts represent an average particle concentration for a given set of conditions for a specific count period, operation, work piece material, operator, and location within a particle controlled area. Changes in any one of these conditions can alter the airborne particle concentration in the critical environment.

The air cleanliness level of a work location can be no better than the cleanliness level of the air approaching the work location. In fact, it will usually be worse. For example, in order to operate at a Class 100 level at a work location, the approaching air will usually have a count considerably less than 100 particles per cu. ft. of air.

With laminar airflow, the lowest number of particles are present in the air flowing into the work location, with the highest number occurring downstream from the work location. Particle counts should be made at various points around, and as close to, the work piece as possible to determine where the highest counts are encountered. It must be assumed that at least some part of the critical work piece will be exposed to the highest counts, for part or all of the time during an operation. The air cleanliness class of the clean air device (not work location), per Federal Standard 2096, can be determined by measuring the airborne particle concentration of air approaching a work location under normal operating conditions.

Usually, the use of rapid response, high sampling rate counters for Classes 100 and 10,000 conditions, will give the best information. Also, this type of rate counter will handle larger particles than slower rate counters. A profile of the work location generally

provides a more accurate picture of the actual particle levels for the entire work location.

APPLICATIONS

Use of the laminar flow clean room, whose essential concept was developed in 1960*, has spread to pharmaceutical companies, hospital operating rooms,

food preparation plants, leukemia-chemotherapy treatment rooms, and a variety of other industries. In fact, it is currently estimated that approximately one million dollars worth of laminar flow equipment is being marketed each year, and that approximately 1000 hospitals have installed laminar flow clean rooms for surgery. Use of this equipment is also widespread in Europe and Japan.

*Laminar flow devices are covered under U.S. Patent No. 3,158,457, Ultra Clean Room, 24 Nov. 1964, W. J. Whitfield; and U.S. Patent No. 3,273,323, Laminar Flow Air Hood Apparatus, 20 Sept. 1966, W. J. Whitfield.

PART 3

THERMAL PROCESS CALCULATIONS AND MODELING

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CHAPTER 8

Analytical Models

"And 'lected it a member of the Fumigated Band."

INTRODUCTION

To minimize the probability of contaminating Mars, the little band of space vehicles that has gone to the planet—the Mariner '64 and '69 flyby vehicles and the Mariner '71 orbiter—had constraints on the aim points and trajectories that could be selected, and limits on the microbiological loads the vehicles were permitted to carry. Viking recently joined the band with similar constraints and bioload limits on its orbiters and sterilized landers.

You will recall from chapter 2, that lander sterility can only be certified inferentially. To ensure that the sterilization process was adequate, NASA used a series of analytical models and computer programs developed specifically for this purpose. These models integrate the estimated microbial burden, its location, its composition, and its thermal resistance characteristics with the thermodynamic properties of the heating medium and the vehicle. They yield the parameters of a sterilization process (a time-temperature curve) that, when properly applied, have a high probability of achieving a sterility level that satisfies the planetary quarantine constraints.

It has been said that a physical phenomenon cannot be understood until it can be reduced to a descriptive equation or model. This chapter describes how NASA's analytical models were developed to: (1) estimate the number of organisms present on a test article; (2) quantify the thermal resistance of the organisms; (3) calculate a specified statistical probability of sterility; (4) thermally model the heating process; and ultimately, combine all of these concepts into an integrated model. The constraints and assumptions that apply to each model, and the concomitant computer programs that were developed are discussed in this chapter.

The development of these models has provided insight into the mechanics of microbial contamination and the kinetics of microbial thermal inactivation. The techniques developed can be used to

determine a heat sterilization process for any item; and they may, with additional work, be extrapolated to sterilization by methods other than heat.

MICROBIAL ESTIMATION FROM ASSAY MODELS

The first step in estimating the required heat sterilization process is to determine the resident population of microorganisms on and within the contaminated article. The necessary duration or intensity of the sterilization process is directly related to the population of organisms. Since the organisms cannot be seen, except by microscopic examination, it is necessary to estimate the population by sampling and culturing. Rarely is it cost-feasible or practical to perform the total area sampling of the article. For instance, it is impractical to disassemble the components of an item for assay purposes, because handling during reassembly will undoubtedly change the biological population count. Therefore, statistical, quality inference techniques are used.

For example, partial area assays are customarily used to infer the total microbial population on an item. This involves the acquisition of surface organisms from a limited area by wiping or vacuuming. These organisms are then counted. The number counted is then extrapolated upward to estimate the total microbial population on the spacecraft. In this process, factors such as the area of the article and the percentage efficiency of the assay are taken into account. The latter is significant, because in each step of the assay process some organisms will not be detected (organism loss). Imperfect sample acquisition, losses during handling, inappropriate culture media or conditions, and individual cells lost on implements or by clumping of the cells can increase this loss. Models that account for these losses have been developed (ref. 198).

Carefully controlled experiments on specially prepared samples have been conducted to quantify the various sources of organism loss, from the test article to the culture count. Results indicate that only 30 to 70 percent of the microbial population present will be reflected in the assay counts, due to sampling inefficiencies and losses. Once the sampling efficiency is determined, conventional statistics can be applied to extrapolate from the assay model an estimate of the total microbial population.

In general, the assays will not yield the same value for all areas, since organisms are usually not uniformly distributed over the test article. Therefore, each sample assay from a specific location will yield a given population count per unit area. The expected value and the statistical bounds of lowest and highest estimates for the total article can be established by using a frequency distribution, i.e., number of organisms per unit area, established for ten to hundreds of area samples. The key to accuracy in this modeling process is curve-fitting the empirical data (number of organisms per unit area) to a mathematical function that represents the probability of achieving a specific sample count. The range of values for the total microbial population is related to the proportion of the total area sampled and the counts per unit area reported from the assay. If many samples are taken, i.e., over 10 percent of the total article area, and if homogeneity of distribution over the surface is indicated by a small sample-to-sample variance, then the estimated extreme values of the total population will differ by less than a factor of 10. If the conventional rules of quality-control sampling currently prevalent in most production organizations are applied, the number of organisms can be estimated with confidence.

Models have been developed to accommodate all of these factors for spacecraft application. A typical representation of this model is presented in figure 66. Similar models can be developed for any assembly or manufacturing process by considering the constraints peculiar to particular assembly operations, environment, and assay procedures.

THE CHRONOLOGICAL PREDICTION MODEL

The procedure described for microbial population estimation is valid only for the time period in which the assays were taken, because organism populations change with time. To accommodate this behavior, it

is necessary to project the estimate of microbial population for future times, unless assays are to be performed continuously. If the assays are deferred until immediately prior to the sterilizing process, then the bioload will not be known soon enough to select the process parameters. In most cases, it requires up to five days to sample, incubate, and count the assays. Therefore, a model was needed to project the population estimate for future time.

The required population dynamics model is not a simple one, because in any given time many external influences affect the microbial population. Organisms can accumulate by precipitation from the air, from tools and, most significantly, from personnel. Counter to these increases, there may be some organism removal or deaths due to environmental stresses such as wind, sunlight, cleaning procedures, and other factors.

To construct an analytical model to account for such diverse circumstances required that each operation, which could influence the microbial population, be quantified as to its rate of accrual or reduction in organism population. The required parameters, such as transfer of organisms from tools, the air, and from personnel, were evaluated by controlled testing of a variety of sample strips under simulated spacecraft conditions. A detailed sequence of events was developed to include all of the environments and operations that a spacecraft undergoes during assembly. Population change rates were estimated for each step based on exposure time to each environment and for each operation. With this technique, the population estimate from the preceding assay model could be extrapolated to project future population estimates. This model for a "typical" spacecraft assembly cycle is described in detail in reference 198. The validity of the model was substantiated for Mariners '69 and '71. These spacecraft were not sterilized, but the total bioload was estimated. Assay data compared favorably with the predictions of the model modifications, which accounted for differences in actual assembly and test operations from that of "typical" assembly. A comparable model could be constructed for any other object and its corresponding assembly or handling operations.

THE THERMAL RESISTANCE OF ORGANISMS

Having developed a method to estimate the total number of organisms on an object prior to applying

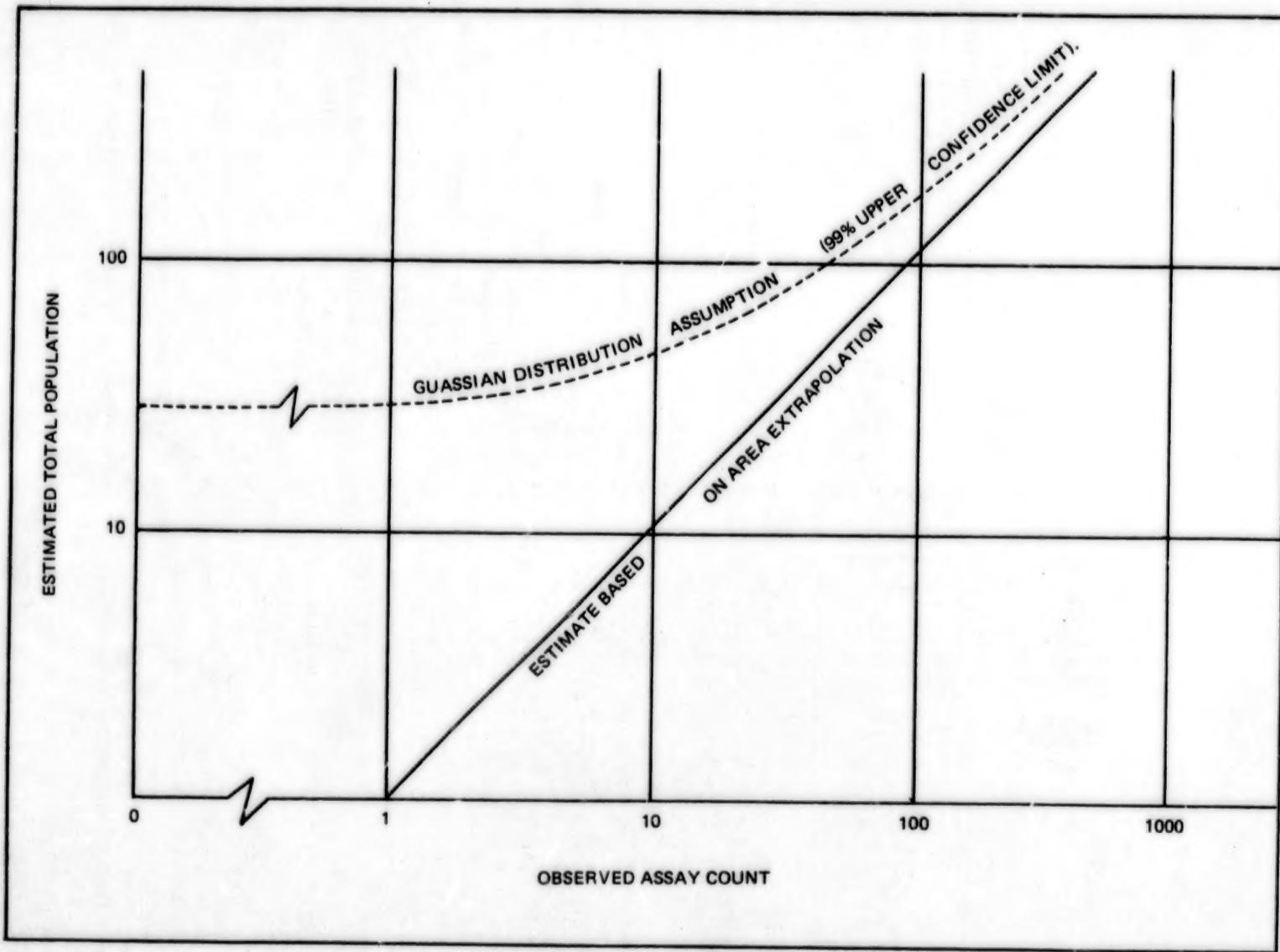


FIGURE 66. — Typical confidence limits.

heat sterilization; it was necessary to determine how fast the organism population would be reduced in number, or die, during the heating process. The concept of the thermal death rate of microorganisms was presented in chapter 2. The model for determining the thermal death rate is based on experimental evidence and certain assumptions.

The generally accepted assumptions are:

- The deaths of individual organisms within a population are independent events.
- The level or intensity of the sterilizing environment is constant.
- Only a single species of organisms is considered.

Equations have been developed as a result of these assumptions. For example, death rate is assumed to be exponential, i.e., the probability of a given organism's surviving (p) t hours at temperature T is given as

$$p = 10^{-t/D_T} \quad (1)$$

The decimal reduction time (D_T) is the time at temperature T necessary to reduce the probability of survival of the population to 0.1. The D_T is different for each species and each exposure environment, and is affected by culture conditions and the physiological state of the population; for example, age.

Since an organism can either live or die after heat exposure, the binomial probability distribution function is applied. To determine the probability of survival (P) of n survivors in an initial population of N organisms, the following equation is used:

$$P = \left(\frac{N}{n}\right) p (1-p)^{N-n} \quad (2)$$

where p is the individual survival probability from equation (1).

The expected number of survivors (\bar{N}) after time t is

$$\bar{N} = Np = N \times 10^{-t/D_T} \quad (3)$$

In actual cases, the survivors are always integers, but the expected number is not an integer or a probability. The expected number \bar{N} is the average number of survivors per item, if the process were simultaneously applied to a large number of test items.

The probability that one or more microorganisms survives time t at temperature T is one minus the probability that none survive. This probability can be

determined from the binomial function [eq. (2)] for $n = 0$. This computation results in P (one or more survivors) = $1 - (1 - p)^N$. The probability of one or more survivors can be approximated by a series expansion of the P function; thus,

$$P (\text{one or more survivors}) = Np + \frac{N^2 p^2}{2} + \dots \quad (4)$$

For Np much less than one, the probability of survivors is equal to the expected number of survivors since the $N^2 p^2$ term becomes insignificant. The error in dropping this term is small (less than 0.5 percent for $Np = 0.1$), so P (actual survivors) is always slightly less than the expected number of survivors; therefore, the approximation is slightly conservative.

The probability that sterility is not reached in the process is the same as the probability of one or more survivors:

$$p (\text{nonsterility}) = N \times 10^{-t/D_T} \quad (5)$$

for $N \times 10^{-t/D_T}$ much less than unity.

It must be realized that absolute sterility cannot be assured [\bar{P} (survivors) = 0] for any but an infinite time process. In practical applications, a preselected risk of nonsterility is accepted and the process time is computed to match this risk. Obviously, when equation (5) is extrapolated over many powers of ten in population numbers, the behavior of the simple exponential model can be expected to depart from that of the actual process. At present the accuracy in extrapolating to a very small survivor fraction is the subject of considerable controversy. Unfortunately, data are difficult to develop at the low population levels because of the impracticability of performing the large number of tests required to verify such small population fractions with confidence, and because of the high risk of accidental test sample contamination during assay when very few survivors are expected.

Influence of Process Temperature

Assuming that thermal death varies exponentially within a particular temperature range,

$$D = D_{T_0} \times 10^{\frac{T_0 - T}{z}} \quad (6)$$

where D_{T_0} is the rate at a reference temperature T_0 , T is the sterilization test temperature, and z is an empirical constant peculiar to a species of organism. (The D-value and z-value are two specific parameters that describe and compare the thermal resistance of microorganisms and which are used in designing the sterilization process. (See chapter 2.) The lower limit of applicability is usually between 80°C and 100°C. Small changes in temperature can create vastly differing rates of sterilization. For example, the z-value for *B. subtilis* var. *niger* spores at $T = 125^\circ\text{C}$ is 21°C. Therefore, a temperature change of 125°C to 146°C reduces the D-value by a factor of 10.

Influence of Environmental Closure

Not all microorganisms in or on an object to be dry-heat sterilized are exposed to the same sterilizing environment. Those on the immediate surface will be exposed to the dessicating effects of the dry oven gas (air). Other organisms may be in joints, cracks and interior cavities, and will not be exposed to the same environment as the surface population. These conditions are treated in the model by the definition of three classes of environmental closure: encapsulated, surface, and mated.

An organism encapsulated within a solid medium, which is essentially impervious to water-vapor diffusion, exhibits a characteristic D_T ; i.e., required sterilization time at temperature T . Organisms on the surface can freely diffuse vapors into the heating environment and characteristically exhibit a different D_T -value. Organisms neither buried nor exposed, but capable of some vapor diffusion with the environment, exhibit a third type of D_T -value. The latter are organisms lodged in mated interfaces, joints, screw threads, etc., which are covered but not sealed from the environment.

The variation of D_T with temperature for the three classes of exposure for *B. subtilis* var. *niger* spores accepted by NASA is presented in figure 67. The highest D-values are associated with encapsulated organisms and the lowest D-values are associated with surface organisms—i.e., the former require the longest sterilization time at temperature T and the latter require the least time.

As discussed in chapter 2, the rate of water transfer to and from cells is the principal factor influencing the death rate of organisms. In turn, the ability of cells to transfer water is dependent on their environ-

mental exposure. Figure 68 presents D_T results for surface-exposed *B. megaterium* spores that were heated in various relative humidity environments at several temperatures (ref. 199). At near zero water activity (100% relative humidity), the D_T is characteristically lower than at activity values near 0.4. At water activity beyond 0.4, D_T again becomes lower. A slight water activity (a_w) change from 0 to 0.3 (indicated in figure 68) is a factor of change greater than 10 in D_T . The large effect of water activity on D_T values indicates that large errors in estimating the required sterilization time can result from inaccurate determination of the relative humidity. Similar curves for encapsulated organisms have been reported in chapter 2. (See figure 13.) In this case, the water in the environment prior to the encapsulation is most important in affecting the D_T -values of the microorganisms.

In efforts to describe the influence of water transfer on the destruction rate, some investigators have developed physiochemical bases to explain the thermal death process. A chemical approach relates the death process to the reaction rate of the chemical compounds, including water in a vital cell "strate" (ref. 200). In another approach, the diffusion process for water migration within and out of the cell is analytically modeled, and the observed behavior of organisms is matched to the coefficients of the model (ref. 201). This approach is similar to the simple exponential model, with the exception that the time required for internal water diffusion introduces a time-lag factor into the solution. These models rely on experimental data for definition of the various coefficients in their equations and their primary objective is to relate the biological destruction process to a more understandable physical process.

THE PROBABILITY OF SURVIVAL

In most applications, the desired surviving microbial population will be zero; i.e., the article will be sterile. This condition is only possible for an infinitely long process time; therefore, it is necessary to establish an acceptable probability value for microbial survival; i.e., the probability that one or more organisms has survived the sterilization process. This probability number must be less than one. In most instances it is not important to know precisely how many organisms survive, but rather whether survival of any is possible. A typical value of the probability

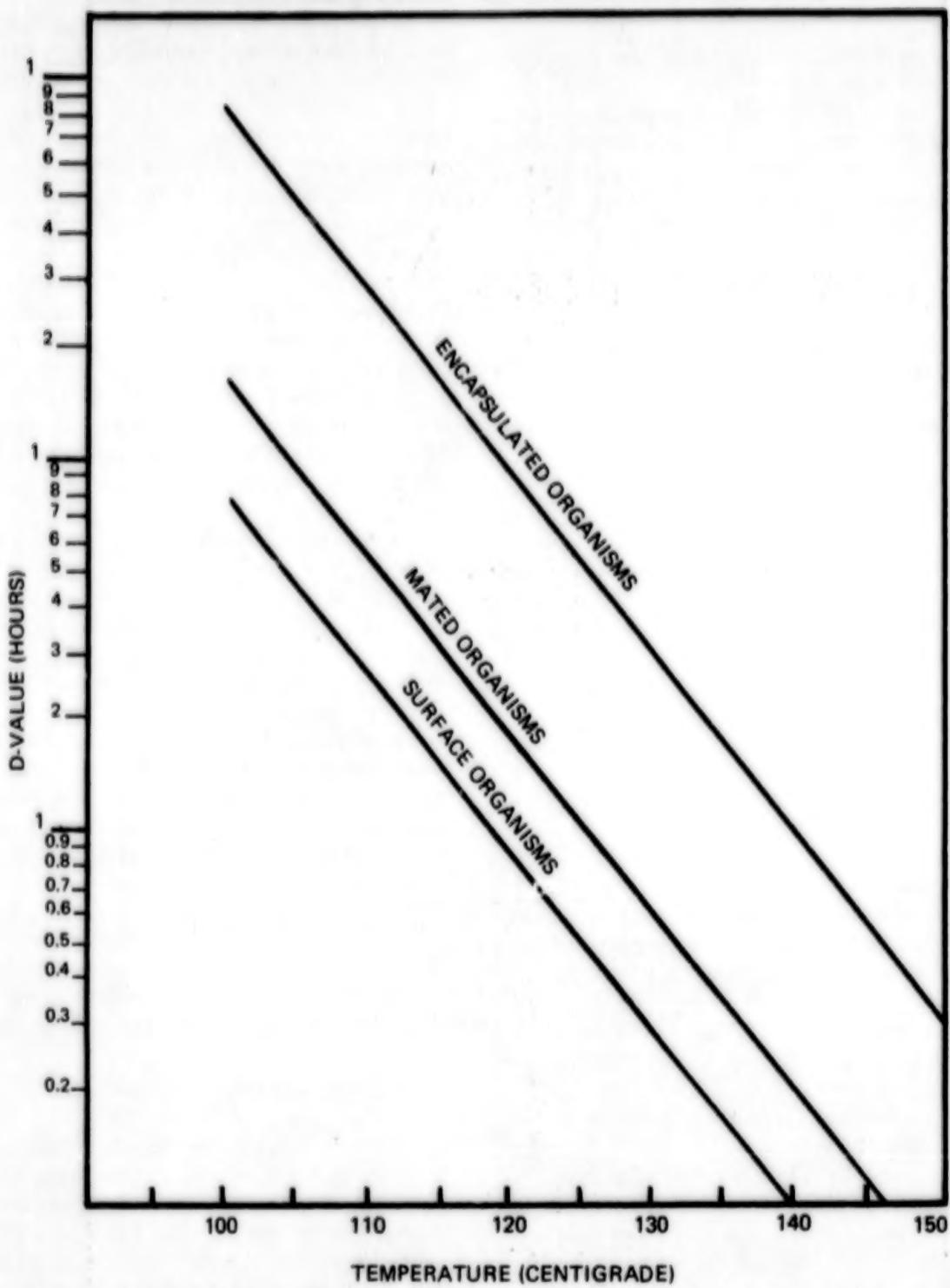


FIGURE 67. - D-value as a function of temperature for *Bacillus subtilis* spores.

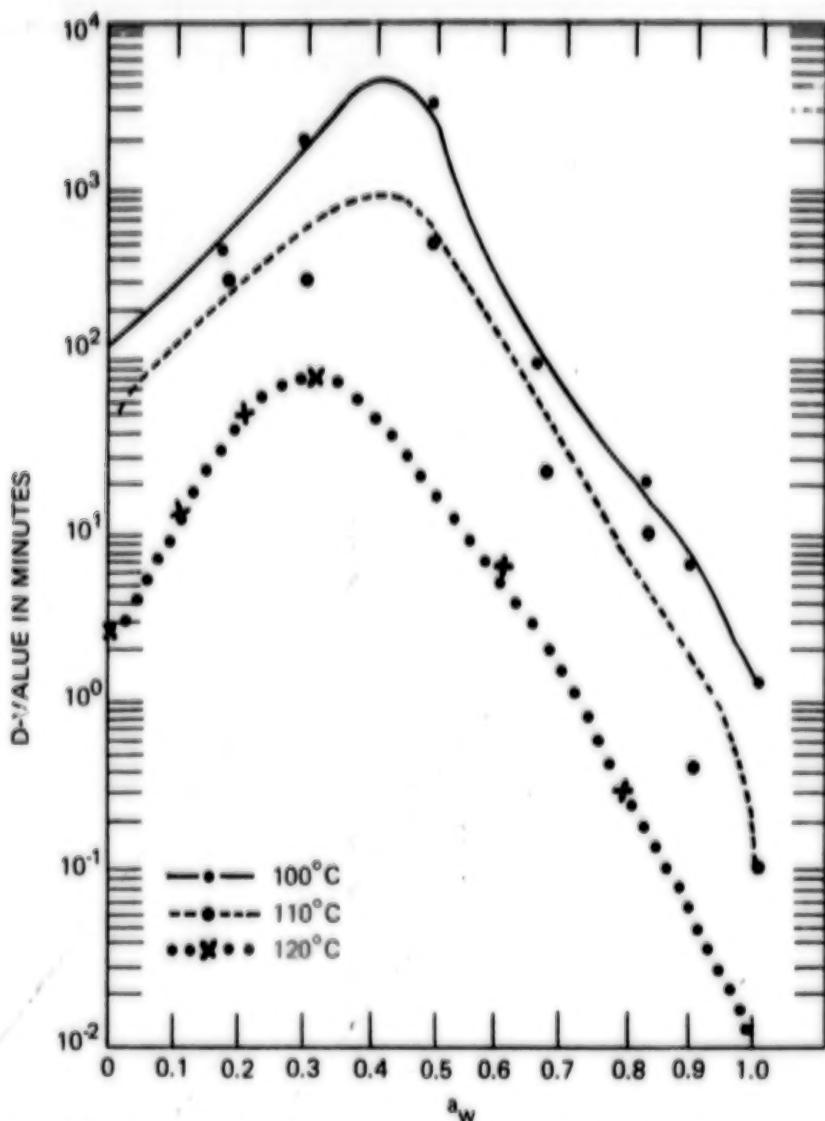


FIGURE 68. – D-values (time required to reduce the population by 90%) of *Bacillus megaterium* spores as a function of water activity.

of a survivor is 10^{-4} . This value represents a cumulative probability that there will be surface, mated, or encapsulated organisms that will survive on any part of the object to be sterilized—including those organisms located away (remotely located) from the most readily heated area.

The probability of a survivor has been discussed previously in this chapter, but is reiterated here to illustrate that P is an accumulation of individual survival probabilities from the various contaminated areas and environmental conditions. The probability of a survivor (i.e., nonsterility of the article) is:

$$P(\text{survivor}) = \sum_{\text{Area}} \sum_{\text{Mated, Surface, Encapsulated}} N \times 10^{-t/D_T}$$

where each element of the double sum is sufficiently small, so that its independence may be assumed.

The selected survivor probability encompasses all areas of the test article and does not treat only the surface organisms. In fact, because the D_T -values displayed by the encapsulated organisms differ from those of the surface organisms, a relatively small number of encapsulated organisms may dominate the

process parameter selections. For the *B. subtilis* var. *niger* spores, the D_T -times are approximately 1/2 hour, 1 hour, and 5 hours, respectively, for surface, mated, and encapsulated organisms at 125°C. In 5 hours (at 125°C) the population of surface organisms is reduced by 10^{-10} ; the population of mated organisms is reduced by 10^{-5} ; but the population of encapsulated organisms is reduced by only a single factor of 10, that is, by 10^{-1} . Therefore, unless the surface population is massive, it will not represent as large a risk of nonsterility as the lower populations of the more resistant, encapsulated organisms. For the purpose of reducing the terminal sterilization time for spacecraft, the encapsulated bioload is significantly reduced prior to terminal sterilization by subsystem or component sterilization processes that are based on the greater D_T -value. Thus, the terminal sterilization process must only kill the microorganisms on surface and mated areas, where lower D_T -values are applicable.

THERMAL-PROPERTIES DEPENDENCE OF THE MODEL

Up to this point the discussion of the modeling of thermal lethality has focused on the organism, and its location, population, and vulnerability. Also of significance are the physical characteristics of the test article and the thermal process oven. First consider the test article. If it is other than a very small and homogeneous, spherical metallic block, the heating process in certain zones will lag significantly. If it is made of loosely bound, metallic components, some parts may take many hours to equilibrate when exposed to a new ambient temperature. Organisms located in and on these thermally remote zones will not be exposed to a very harsh thermal environment compared to organisms on the outermost surface. To accommodate this potential disparity of exposure, the transient thermal behavior of the test article is modeled analytically. This analysis is done by applying the principles of engineering heat transfer.

The test article or spacecraft is arbitrarily divided into analytic zones. Each zone has a specific set of thermal properties that will cause it to behave somewhat differently from neighboring zones. If the test article is analytically subjected to a temperature-time profile characteristic of a thermal lethality process oven, then the transient temperature-time behavior of the separate parts of the article can be estimated.

In some cases, the estimation of physical properties and interface characteristics of the zones on the article will be imperfect. In such cases, it may be desirable to instrument a test article with thermocouples (sensitive temperature-sensing devices) and subject it to a transient thermal process. The behavior of the actual transient temperature of a zone, when compared with the estimated behavior, can generally be made to agree by adjusting the values of the thermal parameters of the model. This adjustment was made for the CSAD-FM program (discussed in chapter 2). The estimated thermal characteristics of several possible property value combinations were computed. By comparing the limited data of a few tests on instrumented hardware, the predicted and actual temperature profiles were made to be coincident (within a few degrees C) over the entire range of temperature and time.

In the development of a transient thermal model, it will be discovered that certain zones of the test article will severely lag the oven temperature characteristic. The alternatives appear to be either to raise the oven temperature higher, or to allow for a longer time to equilibrate the temperature throughout. In practice, a combination of both is used, but not to the degree that may be anticipated initially. In actuality, the death of microorganisms occurs at temperatures lower than the equilibrium temperature of the oven and, in addition, the lethality in these insulated zones continues long after the oven heat is turned off. The same zones that lag in the heating portion of the temperature cycle also lag in the cooling portion. An analytical procedure to integrate the biological factors with the thermal model analysis was developed to account for these lags, and a technique was designed for quantifying the heat sterilization process.

THE INTEGRATED LETHALITY MODEL

To compute and verify that the desired probability of sterility is reached requires that the lethality process be treated for each zone of the test article, for each category of organism (surface, mated, or encapsulated), and for the specific thermal process that each individual zone experiences. This treatment has been accomplished by establishing a bookkeeping system of the pertinent parameters and by computing the cumulative probability of survivors (see refs. 202 and 203). Accommodating thermal transients in the

lethality-process calculation is accomplished by treating the complete process as the sum of a series of short processes, each of which contributes to the overall lethality in the proportion that is dictated by the D_T -value and time appropriate to that step in the process.

Several options are available for use in the integrated lethality calculation. One such option is to divide the process time into a sufficient number of intervals so that temperature is essentially constant at each interval. The total logarithmic reduction in microbial population is additive, since the increments are sequential. The total reduction is $\Sigma (dt/D_T)$, where D_T is the decimal reduction time at temperature T for the i'th time interval (i.e., at a given time). If the interval of time (dt) for each incremental process is allowed to approach zero, i.e., infinitesimal intervals, the sum can be replaced by the integral:

$$\text{Total } \log_{10} \text{ reduction} = \int \left(\frac{dt}{D_T} \right)$$

This expression is exact, but before it can be used, temperature T must be specified as a function of time. Although it is theoretically possible to measure temperatures as a continuous function of time, temperatures are generally given only at discrete points; for example, at 15-minute intervals. Therefore, an assumption is made about the variation in the temperature-time relationship between successive increments. This approach is based on the logic that the actual curved temperature-time history of an article to be sterilized can be replaced by a series of line segments approximating the exact function to any degree of accuracy desired. Therefore, it is possible to predict the total logarithmic reduction resulting from a transient process (either increasing or decreasing in temperature) during the time that the item is above the minimum lethal temperature.

The other option of transient lethality calculation is that presented in reference 204. In this method, the lethal rate during the time interval is assumed to vary as a linear function of time. This simplifies the computation somewhat, but is not a precise fit of the actual lethal rate. However, the simplification is justified because for small time intervals, the prediction converges to an exact integral.

In addition to the transient temperature response, it is necessary to associate an initial number of organ-

isms with each thermal zone to determine the number of survivors and, hence, the probability of sterility. A thermal zone is an arbitrary area within or on the item that experiences a common temperature-time profile. The items' thermal zones are defined by the thermal transient model. For a given thermal zone, it is necessary to discriminate among surface bioburden, mated bioburden, and encapsulated bioburden because each category responds differently—i.e., has different D_T -values.

The bioburden in these zones is determined by the assay model and depends on the nature of the material, the handling and environmental exposure, and any prior decontamination. For each thermal zone, the bioburden must be quantified and categorized to derive a quantitative estimate of sterility. The configuration of the item will determine the number of thermal zones which must be treated. It is not always obvious which thermal zone will dominate the process-time selection. A combination of high initial bioburden and low, integrated thermal lethality at a zone dictates the process-time selection.

To calculate sterilization time requires that three factors be provided:

1. Microbial burden (by exposure category) for each lethality zone
2. Predicted time-temperature profiles for each lethality zone
3. Desired probability of sterility.

Both the bioburdens and the time-temperature profiles will only be predictions. Time-temperature profiles can be predicted only by assuming some nominal process sterilization time, since the heat diffusion process is very time dependent.

The microbial burdens and time-temperature profiles from the model can be used to calculate the probability of sterility. This value is then compared with the desired probability of survivors to determine whether an increase or decrease in the nominal process time is needed. A trial-and-error procedure is then used to determine the correct time, by modifying the time at each trial to achieve the required results. It is not necessary to generate new time-temperature profiles at each trial of the analytical model, because time-temperature profiles can be modified to approximate any changes.

If the process calculations indicate that an increase in time is necessary to achieve probability of sterility, then the procedure is as follows. Assume that an increase of one D_T unit for encapsulated

organisms is required; i.e., 5 hours in a specific lethality zone. From figure 69, the oven cool-down starts at 30 hours for the nominal sterilization time, and the nominal time-temperature profile for this specific lethality zone is given by curve A. This curve is translated 5 hours to the right on the abscissa to give curve B. The temperature between 30 and 35 hours is assumed constant. If equilibrium temperature has been reached, this assumption is correct; otherwise it is conservative, that is, the lethality is greater than predicted. To reduce the sterilization time by 5 hours, the cool-down portion of curve A is translated 5 hours to the left on abscissa to give curve C. In general, the resulting curve will have a gap, so that curve C is adjusted to match the nominal curve at 25 hours. This adjustment is made by reducing all temperatures on curve C by the factor that causes the curves to join. The result is curve D. A detailed mathematical analysis of transient lethality is presented in reference 203.

The integrated lethality model is the final tool required to complete the quantitative definition of the dry-heat sterilization process. We now have all the elements required to compute the process parameters (temperature and time) for any article for which the organism population distribution and type are

known, and for which the zonal thermal transient characteristics may be predicted. As an aid to future applications of this approach, several computer programs that embody all of the above-mentioned attributes have been published. More about these programs is described in the next section.

AVAILABLE COMPUTER CODES

Several digital computer programs have been developed to predict process time for dry-heat sterilization of spacecraft hardware. Two of these, based on the logarithmic death-rate assumption described previously, are: the SPAN (Sterilization Process Analysis Network) program (ref. 203), and the SPOR (Sterilization Process Optimization Routine) program (ref. 203). Both provide for transient thermal behavior, i.e., integrated lethality, but use slightly different assumptions regarding the curve-fit function. The SPOR program uses the linear temperature-time curve fit of the thermal transient curve, whereas the SPAN model assumes the lethal rate as a function of time to be linear. In the limit of small intervals of time, the results converge, and the difference in any case is commensurate with the interval size selected in the curve fit. Another difference in the programs is that

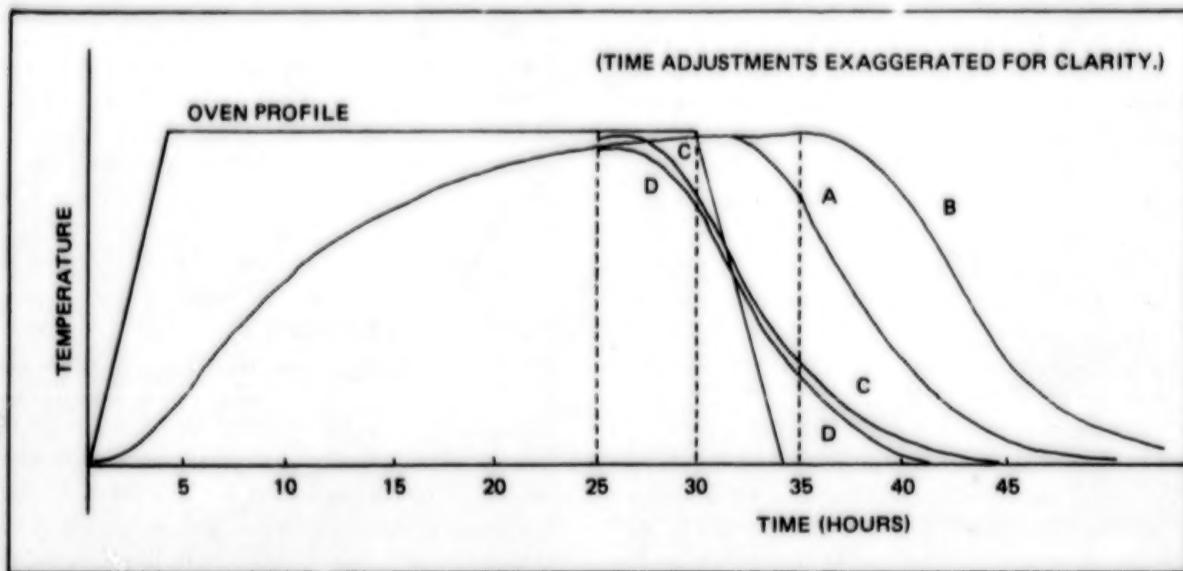


FIGURE 69. — Adjustment of sterilization time.

the SPAN program requires a prior allocation of survival probability to each thermal zone, whereas the SPOR program depends on the process time to yield the sum of zone-survival probabilities for the overall required value. The SPOR program tends to yield slightly shorter process times than the SPAN program as a result of this "optimization" procedure. Both programs are available from the originators for use in nonaerospace applications.

The SPAN program and a variant of it, SPAN C, are available from the COSMIC program library, Computer Center, University of Georgia. Both are written in FORTRAN IV language to use with IBM 7094 computation equipment. SPAN is designed for tape input of operating temperature profiles, whereas SPAN C receives temperature profiles from cards rather than tape. Otherwise the programs are identical. SPAN has the COSMIC reference number NPO-10804, and SPAN C is referenced as NPO-10805.

The basic required inputs for the SPAN programs are: (1) a thermal analysis of the item; (2) the probability of survival (level of sterility) that must be achieved at the end of the cycle; (3) the microbial heat-resistance characteristics; and (4) the number of microorganisms present at the time of sterilization.

The program has considerable flexibility in the values that can be assumed for the basic inputs. The temperature profiles from the thermal analysis must be input on tape. The required tape input is the output (rows and columns rearranged to meet the format requirements of SPAN input) of a thermal analysis program. A minimum number of 250 profiles can be accommodated, including up to 2000 time points on each profile. Each profile can have a different value for the probability of survival. The microbial heat-resistance characteristics and the number of microorganisms (or, if desired, a fixed value for a given run for either of these parameters) can be assigned. Other parameters, which can be varied, include the temperature at which microbial reduction begins, reference temperature, and tolerances. Also available are several tape search options and an alternate computation technique to assess microbial reduction.

COMPUTER IDENTIFICATION PROGRAM

Another available mathematical model, which is not applicable to dry-heat sterilization but which is of great interest in the field of microbiology, is the

computer identification of microorganisms developed by Sandia Laboratories for NASA (ref. 205).

The objective of this program is to systematize the identification of microorganism colonies isolated from assays of spacecraft. To perform this classification, the results of many individual tests (up to 64) are performed on the colony members. The test results are dichotomous; that is, they yield alternative answers. These results are entered onto a standard test format card. Through machine sorting of a colony's response to the many tests, the colony can be classified as one of 95 possible organism categories. This automated identification approach greatly reduces the time required to identify microorganism isolates. In application, the approach was cross-checked against strictly classical manual methods of identification and was found to be highly successful. This computer identification approach could be of value in future application of automated microbiological laboratory procedures.

THE VIKING EXPERIENCE

The ultimate test of any analytical model is its application to a real situation. For the models described in this chapter, this ultimate test came with the sterilization of the two Viking Landers. The analytical models developed to estimate the number of organisms on or inside a test article were used to determine the microbial load of the Landers. The thermal models were used to describe the thermal response of all intricate parts of the Landers. The microbial thermal inactivation models were used to determine the rate by which the Landers' spore population would be reached during the heating process. The statistical models for estimating the probability of survival were used to assure that the specified level of sterility was achieved, and the digital computer programs were constantly working to both predict and simulate the sterilization cycles.

The actual sterilization of the two Viking Landers took place in two especially built ovens at the Kennedy Space Center on June 15 to 17, 1975 and June 20 to 22, 1975. The sterilization cycles for each of the two Landers are shown in figures 70 and 71. Shown in these figures are time-temperature curves for: (1) the Radioisotope Thermoelectric Generators (RTGs), which are the hottest components in the Lander body; (2) the oven gas, which initially represents the driving heat source but which reduces to the

STERILIZATION CYCLE OF VLC-1
JUNE 20-22, 1975

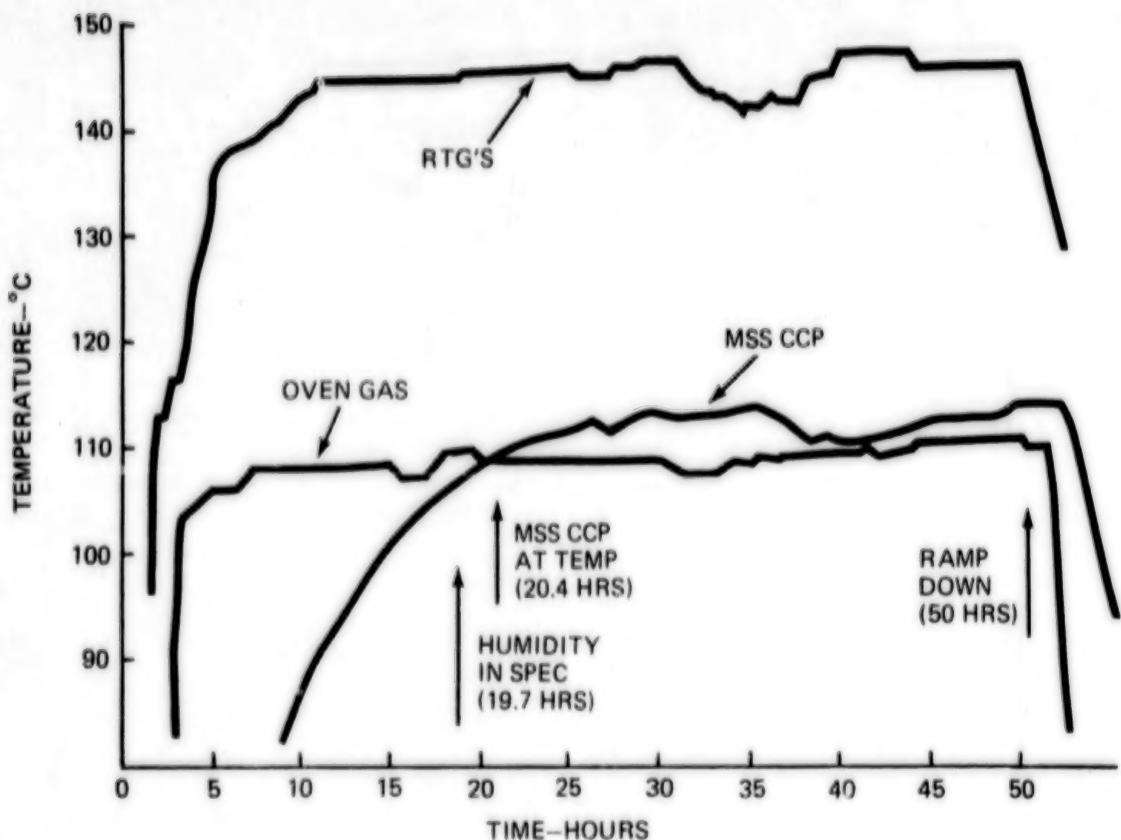


FIGURE 70. — Sterilization cycle of VLC-1.

coldest limit once stable conditions are reached; and (3) the coldest contaminated point (CCP) of the mechanical subsystem (MSS) of the biology instrument package, which is typical of the components in the Lander body. Throughout the sterilization cycles, strategically positioned sensors measured temperature and humidity while appropriate instrumentation recorded and printed the data. The sensors were calibrated before and after the sterilization process and all measurements were adjusted accordingly.

The application of analytical models to the complex Viking situation was by no means a simple and direct process. It required numerous repetitions, many modifications, and the sustained effort of many dedicated individuals to fit the models to each phase of the Viking problem. However, this effort would

not have been successful, if the models were not adaptable enough to permit their fruitful application. Another key factor in the success of the Viking operation was the invaluable experience gained in developing the original models. It was this experience that guided the always-difficult transfer from theory to practice, and which led to sound real-time decision making—an absolute must in the aerospace field.

Among the problems encountered in applying the analytical models to the Vikings was the incorporation into the models of new information which surfaced during the development of the Viking Project. A specific example is the dry-heat resistance of naturally occurring organisms. The existing analytical models, particularly those addressing the microbial death rate and probability of survival, were based on

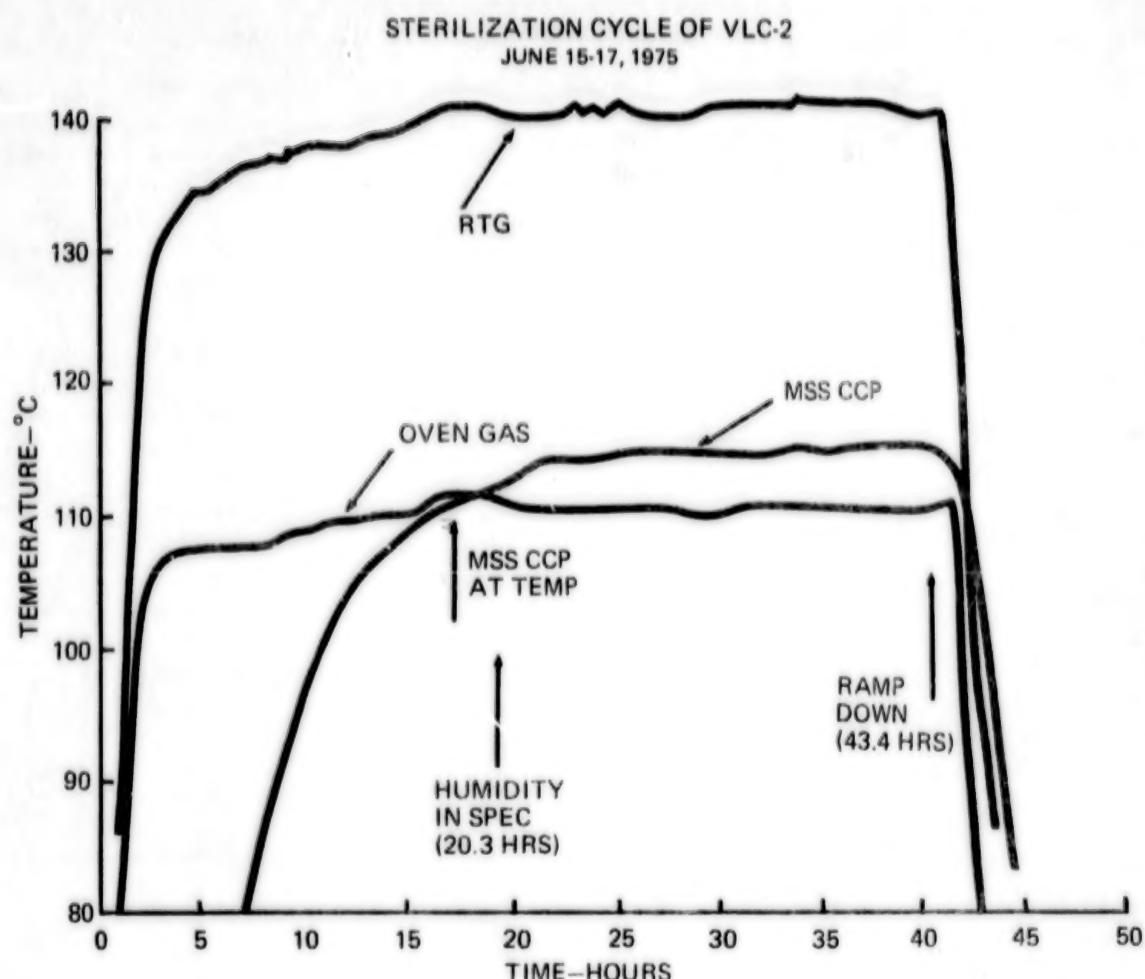


FIGURE 71. — Sterilization cycle of VLC-2.

the D_T -values of *B. subtilis* var. *niger* spores that early research had established as the standard test organism on the premise that their heat resistance was typical of spacecraft resident organisms. Further, these models were developed on the assumption that microbial death rate was exponential—an assumption supported by test results with the standard organism. However, more recent research (recounted in chapter 2) on the dry-heat resistance of naturally occurring microbial populations questioned both the premise and the assumption. Results of some of this research—conducted by the Planetary Quarantine Laboratory (PQL) at the Kennedy Space Center—indicated that there exists a “hardy” microbial subpopulation, whose resistance to dry heat is far greater than that of the adopted standard organism, *B. sub-*

tilis var. *niger*. Later these results were independently verified by soil experiments conducted at the Food and Drug Administration (FDA) Laboratory, Cincinnati, that yielded similar findings. Further verification of the PQL results was obtained when experiments at FDA, Cincinnati—using pure microbial populations subcultured from the survivors of the PQL dry-heat cycles—indicated that indeed their heat resistance was higher than that of the standard organism. These studies also indicated that the surviving hardy subpopulation was not further reduced by extending the heat cycle time; i.e., their death rate did not follow the logarithmic death rate model. Instead, the hardy survivors maintained an almost steady survival level until—at a much longer exposure time—they suffered an abrupt and drastic drop.

The impact of these findings was immediately evident. However, before any assessment could be made of this impact and before any steps could be taken to address it, the results of the research had to be properly quantified. Fortunately, the heat cycles used in the research were tailored to fit the anticipated Viking sterilization cycles. There remained two basic questions to be answered: (1) how large is the hardy subpopulation; and (2) how many survive the cycle? To answer these questions, the entire data from the PQL and FDA, Cincinnati, experiments were statistically analyzed and the results were presented at a "Hardy' Organism Conference" held at Ames Research Center on November 18 to 19, 1974. The statistical analysis, summarized in ref. 200, indicated that for the heat cycles used, a fraction (1×10^{-4}) of the total initial population survived, and that of the initial population 1×10^{-3} were hardy organisms.

These results clearly point out that in estimating the integrated lethality of a given dry-heat sterilization cycle, there exists a base below which one cannot use the heat resistance of the standard organism to compute further microbial reduction. Thus, in predicting and assessing the effectiveness of the Viking Lander sterilization cycles, the logarithmic death-rate models and the statistical models for calculating the probability of survival, were truncated at the level of the established surviving fraction. The assessment indicated that the sterilization cycles for the two Landers were successful in attaining the level of sterility necessary to comply with the imposed planetary quarantine requirements.

APPLICATIONS

The knowledge of microbial death kinetics, embodied in the various models described here, allows for the development of tailor-made sterilization cycles for nonaerospace applications as well. The same principles and approaches that have been applied to the dry-heat process models and which have proven so effective in the complex undertaking of the sterilization of the two Viking Landers, can also be applied to the development of comparable models for other forms of sterilization such as chemical thermoradiation or moist-heat sterilization. The principal value of using such modeling is that optimal conditions, tailored to the test article requirements, can be defined. These optimal conditions will yield the desired sterility level and will be consistent with constraints of minimal process time or temperature. The model approach to sterilization process prediction will be particularly helpful whenever the thermal tolerance of the article is limited or the process time is critical.

*There's not a Micrococcus in the garden where
they play,
They bathe in pure iodoform a dozen times a
day
And each imbibes his rations from a Hygenic
Cup
The Bunny and Baby and the Prophylactic Pup.*

Strictly Germ-Proof, Stanza IV.
Arthur Guiterman (1871-1943)

Note on the Poem

Arthur Guiterman was a humorous poet, a playwright, and lecturer who wrote the poem, "Strictly Germ-Proof," in the 1930s. It has since become a classic in the field of microbiology. The poem was used in this review because the authors believed it to be somewhat analogous to the problem of spacecraft sterilization. If the Antiseptic Baby and the Prophylactic Pup are taken to be NASA and the aerospace industry, respectively, then the Bunny becomes the spacecraft that must be sterilized to protect the Earth

and other planets. During the past decade, NASA has investigated the potential of all techniques alluded to in the poem for achieving the required spacecraft sterilization. We trust that NASA was successful as the Baby and the Pup in that its Bunnies, the Viking Landers, were properly sterilized, and will continue to perform their mission in a manner that will bring credit to NASA and the many investigators who have dedicated a good part of their lives to this effort.

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